THE ANTIRACHITIC ACTIVITY OF VARIOUS FORMS OF VITAMIN D IN THE CHICK

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ONE FIGURE

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Early in 1930, Hess and Supplee reported that a supplement of irradiated ergosterol equivalent to many times the protective dose of cod liver oil failed to promote normal calcification in the chick. Shortly thereafter, papers by Massengale and Nussmeier ('30) and Mussehl and Ackerson ('30) also indicated that irradiated ergosterol was much less effective in preventing leg weakness in chicks when the potency of the preparations was judged by the usual rat assay technic.

Following the initial demonstration of the species difference in response to the different forms of vitamin D, attempts were made to determine the ratio of antirachitic effectiveness (rat to chick) of irradiated ergosterol to cod liver oil. In the hands of different observers the results have been as follows: Massengale and Nussmeier ('30) 1:100; Steenbock, Kletzien and Halpin ('32) 1:40 to 1:100; Bethke et al. ('33) 1:15 to 1:20; Russell et al. ('32) 1:144 to 1:192; Massengale and Bills ('36) 1:50 to 1:100 or more; Bills et al. ('37) 1:31 to 1:100; Haman and Steenbock ('36) 1:20; Grab ('36) 1:400 in spring, 1:100 to 1:400 in fall or winter and 1:24 to 1:32 in summer.

Various other forms of vitamin D have also been studied from this point of view. Irradiated crude cholesterol has been shown by Waddell ('34) to be equivalent to cod liver oil and superior to irradiated ergosterol in preventing the development of leg weakness in chicks. The antirachitic value of a

semi-amorphous preparation of irradiated 7-dehydrocholesterol for the chick has been reported by Grab ('36) to be one-half to one-third as great in the spring, one-fifth in the summer and one-thirteenth as effective in early summer as for the rat. Koch and Koch ('36) tested on chicks a series of sterol derivatives including irradiated 7-dehydrocholesterol prepared by the method of Windaus, Lettre and Schenck ('35). On the basis of the weight gain, bone ash and appearance, the chicks receiving 10 rat units of irradiated 7-dehydrocholesterol per 100 gm. of diet were more completely protected against leg weakness than those receiving 10 rat units of cod liver oil per 100 gm. of diet. A further experiment revealed that 35 rat units of irradiated corn oil phytosterol were fully equivalent for chicks to 100 rat units of irradiated ergosterol but inferior to 10 rat units of cod liver oil. Bills, Massengale, Imboden and Hall ('37) observed, on the basis of the bone ash values found, that the antirachitic efficiency for the chicken of irradiated 7-dehydrocholesterol, irradiated cholesterol from spinal cord and cod liver oil differed in no significant degree. McDonald ('36) found that rat unit for rat unit, the effectiveness of irradiated 22-dihydroergosterol for the chick more closely approximated that of cod liver oil than that of irradiated ergosterol. Boer, Roerink, van Wijk and van Niekerk ('36), working with a special cholesterol, isolated the natural chicken pro-vitamin. Chemical and biological tests indicated that it was 7-dehydrocholesterol.

Massengale and Bills ('36) reported that the ratio of the antirachitic efficiency of cod liver oil to irradiated ergosterol in the chick for the production of bone ashes amounting to 40% was 1:31, but as the bone ash increased to 47.3%, the ratio increased to 1:220. Thus it appears that the actions of cod liver oil and irradiated ergosterol in the chick are strictly comparable only under specified conditions.

Due to the rather unsatisfactory agreement of the reports in the literature and the fact that many of the preparations which have been tested were relatively impure, it seemed worth while to make a comparative study of the effectiveness

of different types of purified vitamin D preparations in the rat and chick. In this study we have included the pure crystalline forms of irradiated ergosterol, irradiated 7-dehydrocholesterol, a standard cod liver oil, an irradiated cholesterol and Viosterol (A.R.P.I. process) in oil.

OUTLINE OF TEST METHODS

All products, which were tested for their prophylactic values against rachitis in chickens, were assayed in triplicate, using the U.S.P. antirachitic (curative) vitamin D test method on rats.

White Leghorn chicks of a standard breed were received in the laboratory on February 2, 1937, on the second day of life. All chicks were placed in individual wire cages containing water cups. Feeding was begun on the third day of life and continued for 28 successive days thereafter. On institution of feeding, the birds were segregated into twenty-four groups of fifteen to twenty-five chicks each.

The diet fed was a modification of that described by Hart, Kline and Keenan ('31) and consisted of ground yellow corn (57%), wheat flour middlings (25%), crude domestic casein (12%), precipitated calcium carbonate (1%), calcium phosphate (1%), dried baker's yeast (1%), and sodium chloride (1%). The final 2% consisted of cod liver oil (group 24 positive controls), of corn oil alone (group 23 negative controls), or corn oil in which the vitamin D supplement was dissolved. The diet was mixed freshly each third day of the test. The vitamin D supplement was adjusted at such times on the basis of predetermined normal food intakes for this stock breed in order to assure an accurate day dosage of each of the medicaments.

The observations taken during the 28-day period of study included food intake and body weights at 3-day intervals. All birds were sacrificed on the thirtieth day of life. The two tibiae were cleansed of soft tissues and labeled. One was fixed in 90% ethyl alcohol for microscopic examination and

for a permanent photographic record of the degree of calcification present. Microscopic observations included measurements of the width of the metaphyses (standardized micrometer scale) and an examination of serial sections of the bones. The latter test, which will be described elsewhere, permits a rapid evaluation of the degree and character of the calcification present, comparable to that of the standard U.S.P. antirachitic line test. The second bone of each chick was dried, crushed, wrapped in a piece of filter paper and extracted in a Soxhlet extractor for 48 hours with alcohol and an equal period with ether. The bones were then dried at 105°C. to constant weight. They were then ashed for 4 hours at 1000°C. and again weighed.

DESCRIPTION OF THE VITAMIN D PREPARATIONS TESTED

An ample volume of cod liver oil for completion of all tests was standardized on rats, using the U.S.P. antirachitic vitamin D test methods with the U.S.P. reference oil. This product, which assayed 100 units per gram, was also checked with the international vitamin D standard.

The crystalline irradiated ergosterol (vitamin D_2) used was prepared by the method of Windaus, Linsert et al. ('32). The product had a formula of $C_{28}H_{44}O$, a melting point of 116° to 117°C., and maximum absorption spectroscopically at 265 mμ. The specific rotation of the crystals in acetone dissolved in 1, 3-propanediol was $(\alpha)_D^{20} = +81.5°$. Vitamin D_2 is insoluble in water but soluble in organic fat solvents.

The crystalline irradiated 7-dehydrocholesterol (vitamin D_3) used was prepared by the method of Windaus, Lettre and Schenck ('35). This product had a formula of $C_{27}H_{44}O$, a melting point of 82° to 84°C. Its absorption maximum was at 265 mμ and the specific rotation in acetone was $(\alpha)_D^{20} = +83°$. The crystals are practically insoluble in water but soluble in organic fat solvents.

The crystalline vitamins D_2 and D_3 and their physical constants were furnished through the courtesy of Dr. O. W. Barlow of the Winthrop Chemical Company, Rensselaer, New York.

The irradiated crude spinal cord cholesterol in oil was furnished by Dr. J. Waddell of the Acetol Products, Incorporated, of New Brunswick, New Jersey. The potency of the product (lot no. 4499) was 10,000 U.S.P. vitamin D units per gram.

The Viosterol (A.R.P.I. process) in oil was a vitamin D concentrate with a potency of 1,000,000 U.S.P. units per gram in sesame oil. This preparation was described as "a solution in oil of ergosterol activated by the action of low speed electrons produced by suitable controlled electrical means." (Am. Med. Assoc. Council on Pharmacy and Chemistry, '37). The product was furnished through the courtesy of the Research Laboratories of the American Research Products, Incorporated, of Minneapolis, Minnesota.

RESULTS

On the basis of the U.S.P. antirachitic vitamin D test, crystalline vitamin D_2 assayed 0.025 microgram per unit, or 40,000,000 units per gram. Crystalline vitamin D_3 under similar conditions corresponded exactly in antirachitic potency to vitamin D_2. The spring and early summer day dose of the semi-amorphous powdered product obtained from irradiated 7-dehydrocholesterol was reported by Grab ('36) to be 0.06 γ or approximately 17,000,000 units per gram. A product similar to that tested by Doctor Grab, obtained from Prof. H. Hoerlein of the Pharmaceutical Division of the I. G. Farbenindustrie, had a vitamin D potency, according to our assays, of 24,000,000 units per gram. The vitamin D_3 tested in these studies was the purified crystalline product.

The results of our assays of several vitamin D preparations by the prophylactic test on chickens are illustrated in table 1. Under the experimental conditions, a femur ash of 45% or more may be considered normal. The amount of vitamin D necessary to produce normal ash values in chicken bones would appear to be as follows: crystalline vitamin D_3 2.4 I.U. (international unit), cod liver oil 2.2 I.U., irradiated cholesterol 2.7 I.U., vitamin D_2 120 I.U., and Viosterol (A.R.P.I.

TABLE 1

Prophylactic study with vitamin D in rachitic White Leghorn chicks

GROUP NO.	SOURCE OF VITAMIN D	DAILY DOSE		TOTAL FOOD INTAKE AVERAGE	WEIGHT		METAPHYSIS		INDEX OF CALCIFICATION AVERAGE	BONE ASH AVERAGE
		Expected median	Actual median		Original median	Gain median	Width average	Per cent of positive control		
		I.U.	*I.U.*	gm.	gm.	%	mm.			%
1	Cod liver oil	1	0.98	474.5	35.5	410	1.125	168	1.9+	41.17
2		2	2.175	492.5	35.5	484	0.71	106	2.5	45.01
3		3	3.225	504.5	36.0	448	0.72	107.4	3.0	45.57
4		6	6.68	541.0	35.0	529	0.62	92.6	3.05	47.05
5	Crystalline vitamin D_3	1	1.0	492.5	34.0	402	0.885	132	1.9	42.26
6		2	2.38	538.0	36.0	545	0.67	100	2.62	44.96
7		3	3.37	514.5	34.0	505	0.67	100	3.25	45.98
8		6	6.06	546.0	35.0	517	0.60	89.5	3.5	46.52
9	Irradiated cholesterol	1	0.995	453.0	36.0	349	1.61	240	1.5	39.15
10		2	2.22	523.0	35.0	509	1.005	149.4	2.5	42.70
11		3	3.16	509.0	35.0	472	0.71	106	3.15	46.56
12		6	6.37	507.5	38.0	479	0.71	106	3.25	45.52
13	Crystalline vitamin D_3	10	9.35	387.0	36.0	289	3.20	478	1.0	32.34
14		20	20.3	414.6	35.0	367	2.57	384	1.0	38.13
15		50	50.3	481.0	35.0	441	0.967	144	2.38	42.34
16		100	102.5	506.5	35.5	462	0.745	111.2	3.25	44.81
17		200	206.0	506.5	36.0	445	0.73	109	3.25	46.38
18	Viosterol (A.R.P.I. process)	10	10.1	463.5	33.0	344	2.285	342	1.0	33.40
19		20	19.85	457.0	35.0	375	2.29	342	1.76	36.56
20		50	51.0	497.0	35.0	403	1.89	282	2.0	40.83
21		100	106.4	517.5	35.5	412	0.70	104.2	3.0	44.55
22		200	210.0	510.0	34.5	451	0.70	104.2	3.0	45.38
23	Negative control—2% C.O.		0	305.9		284	4.18	—625	—2.0	29.45
24	Positive control—2% C.L.O.		36.3	492.2		468	0.67	100	+3.5	46.12

process) 150 I.U. The efficiency ratio of cod liver oil to Viosterol (A.R.P.I. process), rat unit for rat unit, in the chick is 1:63. This value is between that reported by Russell, Taylor and Wilcox ('34) and that of Bethke, Record and Kennard ('33). These groups of workers based their comparison on the minimum protective dose. Massengale and Bills ('36) consider a bone containing 46 to 47% of ash as normal according to their technic. For bones containing 46% ash they find the ratio of cod liver oil to irradiated ergosterol to be 1:74 which is in good agreement with the present results.

The results obtained in these experiments are not as clear cut as might be desired. It will be noted that when 1 unit a day of cod liver oil, vitamin D_3 or irradiated cholesterol was fed, the order of efficiency, according to the bone ash, from high to low, was vitamin D_3, cod liver oil and irradiated cholesterol. When two units were fed, vitamin D_3 and cod liver oil were equal in potency and superior to irradiated cholesterol. At the three unit level, vitamin D_3 and cod liver oil are again equal, but irradiated cholesterol was questionably superior. At the highest dose fed, the order then became cod liver oil, vitamin D_3 and irradiated cholesterol, but it is doubtful whether the differences noted have any significance since the ash values of all bones were within normal limits. However, the results do reveal that the bone ash is not entirely satisfactory for the estimation of the potency of vitamin D preparations in the chicken. Similar criticisms may be made of the use of gain in weight, and, to a lesser extent, of the metaphysis width and direct examination of sections of the bone (modified line test), when used individually as a basis for vitamin D assay in the chicken. It would appear that the use of all four types of data when evaluating the results of vitamin D administration in the chicken would contribute to the accuracy of assays. In the final estimation of the curative day dose, the experimental results were compared with those of the positive control group as to gain in body weight, as well as the width of the metaphysis. Bones with a 3+ index

of calcification, as indicated by a modified form of the antirachitic line test, and 45% or more ash were considered normal. On this basis, the minimal protective dose for chickens appears to be 2.5 I.U. of cod liver oil, 2.5 I.U. of vitamin D_3, 2.7 I.U. of irradiated cholesterol, 85 I.U. of vitamin D_2 and 120 I.U. of Viosterol (A.R.P.I. process). The antirachitic efficiency ratio of cod liver oil or crystalline vitamin D_3 to crystalline vitamin D_2 in the chick then becomes 1:34. The corresponding values for Viosterol (A.R.P.I. process) are 1:48. These ratios are lower than those based on bone ash values alone. These results indicate that cod liver oil, irradiated cholesterol and vitamin D_3 differ to no significant degree as to potency in the chick, rat unit for rat unit. It is probable that at least one of the pro-vitamins found with cholesterol is 7-dehydrocholesterol (pro-vitamin D_3), as has been demonstrated in a special case by Boer et al. ('36). Hence it is not surprising that nearly identical results were obtained with irradiated cholesterol and vitamin D_3. These results, however, cannot necessarily be used as proof that 7-dehydrocholesterol is the only active principle in cholesterol or cod liver oil since it is possible for two chemical compounds to be present simultaneously, each of which have the same antirachitic activity in the chick as has already been shown to be true in the rat.

Crystalline vitamin D_2 on the basis of these data appears to be somewhat more effective in the chicken than the Viosterol (A.R.P.I. process) tested. This observation is in line with the fact that Viosterol is a mixture of compounds, the most important of which is vitamin D_2.

Figure 1 illustrates the average effects of various dosage levels of the several types of vitamin D upon the bone ash. The experimental points fall about two curves which are dissimilar in nature. For convenience in graphing, the abscissa has been multiplied by twenty for the Viosterol (A.R.P.I. process) and vitamin D_2 data.

The response curves for these two groups of compounds, as illustrated by those of vitamin D_2 and D_3 in the chick are

essentially different and cannot be made to coincide by expansion or contraction of the coordinates. These data, although not directly comparable to those of Massengale and Bills ('36), because of differences in technic, confirm the observations of these authors, relative to the divergent results obtained by different investigators, when comparing

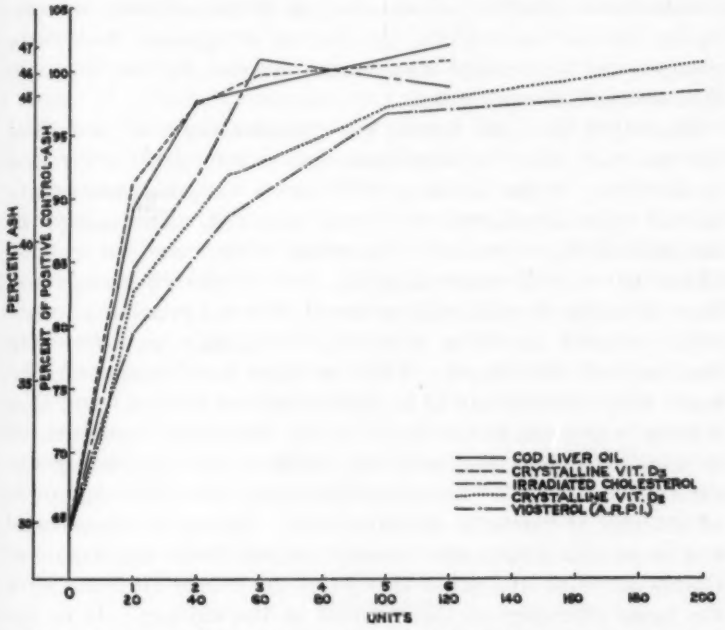

Fig. 1 Response of bone ash to various doses of vitamin D. The abscissa for vitamin D_3 and Viosterol (A.R.P.I. process) is twenty times that of the other substances.

the efficiency of cod liver oil with that of irradiated ergosterol, rat unit for rat unit on chickens. When comparisons of these substances are to be made in chickens, the degree of calcification of the bone must be considered. No comparison can safely be made when the bones have different ash values.

Strictly speaking, these two groups of substances cannot be compared for their antirachitic efficiency in the chick since they apparently function in a slightly different manner physiologically. It may be suggested that irradiated 7-dehydrocholesterol, cod liver oil and irradiated cholesterol can be readily utilized by the organism, while the utilization or conversion of Viosterol (A.R.P.I. process) or vitamin D_2 into a substance suitable for metabolism in the chicken is relatively slow or incomplete. In the rat it appears that these changes can be readily made since vitamin D_2 and D_3 have the same potency per gram.

Russell et al. ('34) found, after medication with cod liver oil, that only relatively small amounts of vitamin D are stored in the liver of the chicken, while much larger amounts are stored when irradiated ergosterol was fed. The assays of the chicken livers were made by means of the usual rat technic. Since the rat will respond equally well to the vitamin in cod liver oil and to irradiated ergosterol, it is not possible to state with certainty the form in which the vitamin was stored in the livers of the chicken. These authors have suggested that more than one vitamin D is present in cod liver oil and that a form is present in the livers of the chicks fed cod liver oil to which the chick responds but which is not effective in the rat. Bills et al. ('37) have concluded that more than one form of vitamin D exists in fish liver oils. However, commercial cod liver oils which may consist of oil from the livers of Gadus morrhua and other species of the family Gadidae have the same efficiency in the rat and in the chicken. It is not improbable that the storage noted in chicken livers after irradiated ergosterol feeding was in the form of vitamin D_2. The degree of utilization or the rate of conversion by the chicken of this compound into a physiologically more active form of vitamin D may then be the controlling factor in the prophylactic or antirachitic effects of irradiated ergosterol in this species.

Bethke et al. ('33) and Russell et al. ('34) have suggested that two or more factors may be present in irradiated

ergosterol, one of which is effective in both the chicken and the rat and the other only in the rat. In the interpretation of the present experiments, this argument does not hold since both the crystalline vitamin D_2 and Viosterol (A.R.P.I. process) were found to have relatively the same potency when tested on rats, as well as on chicks. Bills, Massengale, McDonald and Wirick ('35) also found after assaying seven specimens of irradiated ergosterol on rats, that when the species efficiency ratio was taken into consideration, the antirachitic values of the several products on chickens were identical. Thus it does not appear that the lower antirachitic efficiency of irradiated ergosterol for the chick as compared with the rat is influenced significantly by the contaminants to be found in the various irradiated ergosterol preparations, although these data suggest that the chick utilized crystalline vitamin D_2 more effectively than Viosterol (A.R.P.I. process) per se. Klein and Russell ('31) have shown that the vitamin in irradiated ergosterol is somewhat more efficiently absorbed than that of cod liver oil in the chicken. The governing factor in the low potency of vitamin D_2 for the chick as found by means of the comparative technic appears to be one of utilization, since cod liver oil and vitamin D_3 are correspondingly potent in the rat and in the chicken. Thus it appears that the chemical differences between vitamin D_2 and D_3 are of primary importance in explaining the relative therapeutic effectiveness of these compounds in the chicken.

SUMMARY

1. Crystalline vitamin D_3 assayed by the U.S.P. antirachitic vitamin D test on rats has a potency of 0.025 γ per unit or 40,000,000 units per gram. This is identical with the potency of crystalline vitamin D_2.

2. The minimal protective daily dose for chicks fed a rachitogenic diet was found to be 2.5 I.U. of cod liver oil, 2.5 I.U. of crystalline vitamin D_3, 2.7 I.U. of irradiated cholesterol, 120 I.U. of Viosterol (A.R.P.I. process) and 85 I.U. of crystalline vitamin D_2.

3. The antirachitic ratio of effectiveness of cod liver oil to Viosterol (A.R.P.I. process) rat unit for rat unit in the chick is 1:48. The ratio for vitamin D_3 to vitamin D_2 is 1:34.

4. The reasons for the variations in potency of vitamin D preparations when tested on rats and chicks are discussed.

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THE EFFECT OF ENHANCED IODINE INTAKE ON GROWTH AND ON THE THYROID GLANDS OF NORMAL AND GOITROUS RATS[1]

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In a recent paper from this laboratory (Remington, Remington and Welch, '37) we presented extensive data on the fresh weight, dry matter and iodine content of the thyroid glands of rats reared on a commercial dog food,[3] which has been found to contain 265 micrograms of iodine per kilogram, and which is an excellent diet for growth and reproduction. Since histological examination of sections from many glands revealed a normal 'resting' state, and since these glands were smaller and contained a higher percentage of dry matter than any others we have encountered, and were smaller than any reported in the literature, we considered the values found as typical for normal rats receiving a fully adequate allowance of iodine. It would be of interest, however, to determine whether increasing the iodine content of this diet, either by the addition of iodide or iodine-rich foods, would produce animals with still smaller glands of higher dry matter and iodine content.

In the work of Levine, Remington and von Kolnitz ('33 a) on the production of goiter by iodine deficient diets, the young rats used were produced on Russell's modification of the Sherman breeding ration, various lots of which were found to

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

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[3] Made by Purina Mills, St. Louis, Missouri.

contain from 47 to 72 γ per kilogram of iodine. Although not fully apparent at that time, it appears in the light of later work that this diet produced rats with definitely enlarged thyroids with relatively low dry matter and iodine content. Hence in the work cited, as well as that on the iodine requirement of the rat (Levine, Remington and von Kolnitz, '33 b), the young rats used were already moderately goitrous, and so the technic must now be considered as of a curative rather than a preventive nature. In that work, when diets containing 400 γ per kilogram of iodine were fed, thyroids were obtained weighing 12.6 to 13.1 mg. per 100 gm., containing 28.0 to 28.3% dry matter and 0.228 to 0.304% of iodine, dry basis. The values which we now consider normal for rats weighing between 50 and 150 gm. are 10 mg. per 100 gm. in weight; dry matter 33%; iodine 0.200%. In reviewing the accumulated data of the laboratory over a period of 6 years, we have observed that whenever rats are reared on an iodine deficient regime and afterward placed on diets high in iodine, the glands will tend to return toward normal, but that either they are still somewhat enlarged and low in dry matter, or the iodine content is unusually high. This gives rise to the thought that with hyperplasia induced by iodine deficiency, the ability of the gland to store iodine may be increased, and suggests the desirability of determining the effect of feeding larger amounts of iodine to goitrous as well as normal rats.

EXPERIMENTAL

Following the same procedure employed in previous experiments, the young rats were placed on the various special diets at 3 to 4 weeks of age, and maintained for 5 weeks, after which they were killed by chloroform, the thyroids removed, weighed, and analyzed. Food intake and growth records were kept on all groups. Each group consisted of eight animals equally divided as to sex. In series I the iodine was added to the diet by evaporating an alcoholic solution of sodium iodide on a portion of the diet so as to yield a concentrate containing 35 γ of iodine per gram. This concentrate was then

mixed with the basal diet (ground dog biscuit) in proportions of 1, 2, 4, 8, 16 and 32%, for feeding to groups A to F respectively. The iodine content of the final mixed diets was calculated from the 265 γ per kilogram in the original, and the amount of concentrate added. In series II the iodine was added in the form of ground dried haddock, which contained 29.6 γ of iodine per gram, and which was added at 2.5 and 5% of the diet of groups G and H respectively. This was done because Remington, Coulson and Levine ('36) have found that at lower levels the iodine of haddock is fully utilized by the thyroid.

In series III the rats used were of the second generation of goitrous animals reared on our iodine deficient diet 342 (Remington, '37). A group sacrificed at the beginning of the feeding period yielded thyroids weighing 25.2 ± 1.8 mg. per 100 gm., and of dry matter 22.0%, iodine 0.024% respectively, and hence could be considered fairly representative of rats made goitrous by iodine lack. Groups B′ and C′ parallel groups B and C of series I. Group G′ received the very large dosage of 25% of dried haddock. The results are presented in tabular form.

INTERPRETATION

In series I and II, in which the young rats were reared on the colony ration and hence presumably had normal thyroids from birth, it will be noted that there is no consistent change in any of the constants on the thyroid gland as the iodine content of the diet is increased up to forty times the 3 γ per day furnished by the colony ration. These rats, at the conclusion of the experiment, averaged about 180 gm. in weight, and from the work cited in the first paragraph, the normal values on colony diet alone would be: fresh weight 8.8 ± 0.40 per 100 gm., dry matter 33.3%, iodine 0.200%. The glands of the present series are smaller, and the iodine content higher. However, we have seen some evidence that the thyroids of summer rats, even on the same diet, are smaller than those of winter rats, and in fact a group of ten animals

(group O) continued on the colony ration simultaneously with this experiment in July and August 1937, gave, for thyroid weight 7.4 ± 0.29, for iodine 0.183. It cannot be concluded that the enhanced iodine intake has brought about any changes in the thyroid, with the exception of a possible slight increase

TABLE 1

Effect of increased iodine intake on the thyroid glands of normal and goitrous rats

GROUP	IODINE IN DIET	DAILY FOOD INTAKE	IODINE INTAKE PER DAY	DAILY GAIN IN WEIGHT	FOOD FOR EACH GRAM OF GAIN	THYROID GLANDS		
						Fresh weight milligrams per 100 gm. body weight	Dry matter	Iodine dry basis
Series I. Iodine added as sodium iodide								
	γ per kg.	*gm.*	*γ*	*gm.*	*gm.*		%	%
O[1]	265	11.5	3.0	3.77	3.05	7.4±0.29	34.1	0.183
A	612	11.3	6.9	3.57	3.16	7.4±0.25	32.9	0.233
B	960	11.3	10.8	3.63	3.12	7.5±0.24	31.8	0.213
C	1304	11.1	14.5	3.63	3.05	7.3±0.18	29.1	0.199
D	2344	11.7	27.4	3.69	3.16	8.0±0.38	33.8	0.223
E	4423	12.0	53.1	3.71	3.20	8.1±0.42	34.1	0.195
F	11380	10.7	121.7	3.63	2.96	7.2±0.31	35.0	0.262
Series II. Iodine added as dried haddock containing 26.6 γ per gram								
G	997	12.4	12.4	3.89	3.20	7.9±0.28	33.2	0.185
H	1730	11.3	19.5	3.91	2.90	6.8±0.20	34.0	0.250
Series III. On second generation of goitrous rats								
N.C.[2]						25.2±1.80	22.0	0.024
B'	960	10.0	9.6	3.43	2.91	8.0±0.32	30.0	0.322
C'	1304	10.0	13.0	3.34	3.00	8.9±0.39	29.9	0.288
G'	7600	10.3	78.3	3.69	2.79	8.3±0.22	29.0	0.281

[1] Basal diet without added iodine.

[2] Parallel group at beginning of experiment.

in the iodine content. It is also worthy of note that growth and the utilization of food for growth are not affected by amounts of iodine as high as 50 to 100 times the minimal protective dose of 1 to 2 γ per day, which amount, however, is still far below that which would bring about any pharmaco-dynamic effect.

Series III, in which the iodine enriched diets were fed to rats previously suffering from iodine deficiency, also shows nearly complete recovery as to weight and dry matter content of the thyroids, but unusually high iodine values. We have found in other experiments that diets which contain enough iodine to prevent thyroid enlargement, will not restore normal values in rats already goitrous. In one such experiment iodide was added to diet 342 (iodine deficient) so as to yield 5 γ per each 10 gm. of diet, or 5 to 6 γ per rat per day, and this diet fed for 6 and 14 weeks, respectively, to severely goitrous young rats. Parallel groups were maintained on diet 342 without the added iodine.

TABLE 2

Effects of iodide feeding on the thyroid glands of goitrous rats

	SERIES A CURATIVE PERIOD 6 WEEKS				SERIES B CURATIVE PERIOD 14 WEEKS			
	Number of animals	Thyroid weight per 100 gm.	Dry matter	Iodine dry basis	Number of animals	Thyroid weight per 100 gm.	Dry matter	Iodine dry basis
Before experiment	8	27.0±1.08	% 22.1	% 0.007			%	%
Without iodide	8	25.9±1.57	22.2		10	31.0±1.25	22.2	0.007
With iodide	10	11.9±0.42	28.8	0.195	8	12.1±0.41	28.4	0.164
Normal values		8–10	33	0.200				

Here we did not get abnormally high iodine values in the glands, but neither did we get full recovery as evidenced by weight and by dry matter content. A comparison of the data of the two tables reveals that when relatively large amounts of iodine are fed to goitrous rats, the glands return to normal or near normal in size and dry matter, but contain an abnormally high percentage of iodine; while if the amount given is smaller, yet still well above the preventive requirement, the return to normal is not complete so far as weight and dry matter content are concerned, but may be so as to iodine.

Other cases of abnormally high iodine content in glands which were slightly larger and more edematous than our normals have been encountered in this laboratory, but always

in rats that had a history of more or less severe iodine depletion previous to the iodine feeding period. Reference has already been made to the work of Levine, Remington and von Kolnitz in which the lowest thyroid weight obtained was 11.8 mg. per 100 gm., the highest dry matter 28.3%, but the highest iodine 0.304% in one group of ten rats. One of us spent of the summer of 1937 at the University of Minnesota, where we observed that ten rats from the colony of Dr. C. M. Jackson, killed at 9 weeks of age, yielded thyroids weighing 12.2 ± 0.24 mg., and of 27.4% dry matter and 0.102% iodine content. According to our criteria these must be considered as moderately goitrous and iodine deficient. A parallel group of these animals, transferred to a diet richer in iodine at 4 weeks and maintained thereon for 5 weeks longer, gave values of 9.1 ± 0.42, 31.0 and 0.327 respectively. The average weight of these animals was 157 gm., hence the glands are of normal size, but the iodine value is 160% of normal.

SUMMARY AND CONCLUSIONS

The presence of 265 micrograms of iodine per kilogram in an otherwise well-balanced diet is sufficient to protect growing rats from iodine deficiency as evidenced by changes in the thyroid gland.

Further enhancement of the iodine content of the diet either by sodium iodide or dried haddock, up to forty times the amount present in the original, does not significantly affect the weight or dry matter content of the glands, but may possibly bring about a slight increase in the iodine content.

Iodine in the diet, up to 50 to 100 times the minimal protective dose, does not affect growth or utilization of food for growth.

If iodine enriched diets are fed to rats with enlarged and hyperplastic thyroids, the gland tends to return to normal weight and dry matter content, but such glands can store more iodine than normal ones.

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THE ROLE OF VITAMIN D IN THE CONTROL OF DENTAL CARIES IN CHILDREN

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ONE FIGURE

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Data on nutritional control of structure and stability of teeth are steadily accumulating. The literature has been reviewed at various times (Mellanby, '34; Com. for Invest. of Dental Disease, '36; Boyd, Drain and Nelson, '29). The present paper reports observations, principally on the effects of vitamin D, covering 4 successive years (1930 to 1934). Some of the material has been published (McBeath, '32, '34). It is now possible to correlate all the results and give a clearer picture both of caries incidence as encountered under our conditions and of the effects of vitamin D.

The original plan at the beginning of the 4-year study was to collect data during a school year. On this basis we had relative assurance that the groups would remain intact. During the first year three schools were available, each furnishing one experimental and one control group. The aim was to have twenty to thirty children in each group. As the work progressed it was found that quite a number of children was available during several successive years. This automatically extended the control observation periods over the summer, allowed us to study some of the children both as control and experimental subjects and opened up the question of seasonal effects.

The schools selected were orphanages in and near New York City, permanent homes of the children studied. Two are in the Borough of Brooklyn (St. John's Home and St. Joseph's Female Orphan Asylum); four are just outside the city limits

(Graham School, Hastings-on-Hudson, St. Christopher's School and the Children's Village in Dobbs Ferry, and Leake and Watts Home School in Yonkers, N. Y.). Three schools are conducted on the cottage plan (St. Christopher's School, Graham School, Leake and Watts Home School). The ages of the children ranged from 6 to 14 years.

THE MEASURE OF CARIES INCIDENCE

Various plans for recording carious activity in the teeth have been adopted. Several British investigators reported (Mellanby, '34; Com. for Invest. of Dental Disease, '36) the per cent of carious teeth as a fraction of the total number of teeth non-carious at the first examination and also estimated the intensity of the process by means of arbitrary numerical designations which were summated for the final result. In the present study a plan was adopted which in a sense combines these two features in one score.

Each mouth was subjected to a thorough examination including the teeth and the gums. With regard to the teeth a record was made of quality and color of the enamel, stains, cleanliness and caries, or abnormal conditions. Caries were recorded according to the involvement of the five surfaces: 1) mesial, 2) distal, 3) buccal or labial, 4) lingual, and 5) occlusal or incisal, as first suggested by Bodecker. The present report deals exclusively with the progress of caries as indicated by the increase in the number of surfaces involved in carious lesions. Any fissure deep enough to catch the explorer was recorded as carious. The record, therefore, included every detectable definite lesion of the enamel. Lesions involving more than one surface were outlined on charts according to their location and were scored according to the number of surfaces involved. Fillings were also scored by the number of surfaces involved. The error due to extension by operative intervention is apparently small.

The progress between two examinations is reported as the number of new carious surfaces per 100 days per mouth. In each individual child the number of carious surfaces found was noted on a suitable dental chart, and after the completion

of the year's observations the charts showed the number of new carious surfaces which appeared during each interval. Next on the basis of the number of days between examinations the number of new carious surfaces in each individual was calculated per 100 days. The score for the group was then determined as the mean number of new carious surfaces per 100 days per mouth.

The general plan was to conduct the first examination in the autumn, the second during the later part of the winter and the third final examination late in spring. In the studies of 1931–1932 the mid-winter examination was omitted. The dates of examinations are given in table 1.

Periods between examinations are designated as:

Autumn-winter period, the period between the first and second examinations, usually 100 to 180 days.

Winter-spring period, the period between the second and third examinations, usually 60 to 90 days.

Autumn-spring period, the period between the first and third examinations, usually 150 to 250 days (autumn-winter and winter-spring periods together).

Summer period, the period between the third (spring) examination of one school year and the first (autumn) examination of the following school year.

One year period, the period between an autumn examination of 1 calendar year and the autumn examination of the next calendar year.

Since in the groups here reported the average age and the average number of teeth per mouth are relatively constant and since the groups are of fair size, the results for a group can probably be safely expressed on a mean rate basis in terms of new carious surfaces per 100 days per mouth. This is called the 'score.' Separate classification by age, weight, rate of growth, previous condition of caries and average distribution of deciduous and permanent teeth showed no trends that disturbed the significance of the mean values of the 'score.' A number of interesting findings, similar in part to those of Anderson et al. ('34), are brought out by these tabulations and will be reported separately.

TABLE 1

Data chronologically arranged

SCHOOL		DATES OF EXAMINATION		MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH (871 CASES—270 CONTROLS, 601 EXPERIMENTALS)							
				Number of cases		Autumn-spring		Autumn-winter		Winter-spring	
		Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1930–1931											
(A)	Diet + vit. D [1]	11/7 –3/3 –5/20	11/7 –3/3 –5/20	28	18	0.74	2.12	0.71	2.39	0.77	1.71
(B)	Diet + vit. D [1]	11/21–3/13–5/28	11/21–3/13–5/28	27	11	0.39	1.88	0.53	1.22	0.24	2.87
(C)	Diet + vit. D [1]	11/14–3/17–6/8	11/14–3/17–6/8	17	27	0.49	1.80	0.43	0.93	0.56	3.08
1931–1932											
(A)	Diet + vit. D [1]	12/11–5/20	12/11–5/20	35	18	0.32	1.28				
(B)	Diet + vit. D [1]	12/8 –5/17	12/4 –5/17	26	13	0.33	1.49				
(C)	Diet + vit. D [1]	11/27–5/28	12/7 –5/24	21	18	0.26	1.65				
(X)	Viosterol [2]	12/3 –5/27	12/3 –5/27	21	23	1.56	1.93				
(X)	U. V. light on skin [4]	12/3 –5/27		19		0.27					
1932–1933											
(A)	Diet [5]	12/6 –3/3 –5/12	12/6 –3/10–5/16	37	21	1.55	2.45	1.21	2.38	1.97	2.56
(Z)	Viosterol [3]	11/11–2/28–5/5	11/3 –3/7 –5/9	21	26	1.52	2.92	1.31	1.79	1.88	3.17
(Z)	U. V. light on skin [4]	11/18–3/7 –5/2		27		0.97		0.88		1.13	
1933–1934											
(A)	Milk [6]	9/29–2/8 –4/17	9/25–2/8 –4/17	18	19	1.03	2.32	0.97	1.71	1.14	3.56
(A)	Milk + 400 U. [8]	9/25–2/6 –4/17		22		0.76		0.75		0.78	
(A)	Fresh vit. D milk [10]	9/29–2/7 –4/17		21		1.16		0.98		1.51	

(C)	Milk [6]	9/26–2/26–4/19	9/26–2/26–4/19	19	17	2.86	2.64	2.17	2.35	4.86	3.51
(C)	Milk + 400 U. [8]	9/26–2/26–4/18		22		0.98		0.69		1.86	
(C)	Fresh vit. D milk [10]	9/26–2/26–4/18		24		0.92		0.65		1.72	
(Y)	Milk [6]	10/9 –1/31–4/26	10/9 –1/31–4/26	30	30	1.61	2.73	0.85	2.92	2.64	2.47
(Y)	Milk + 250 U. [7]	10/9 –1/31–4/26		30		1.59		0.99		2.39	
(Y)	Milk + 800 U. [9]	10/9 –1/31–4/26		28		0.39		0.22		0.63	
(Z)	Milk [6]	10/16–2/21–4/23	10/11–2/23–4/24	28	29	2.17	3.09	1.53	2.45	3.51	4.54
(Z)	Milk + 250 U. [7]	10/18–2/23–4/24		24		1.09		1.72		2.57	
(Z)	Milk + 800 U. [9]	10/18–2/23–4/24		30		0.64		0.70		0.50	
(Z)	U. V. light on skin [4]	10/11–2/21–4/24		26		0.96		0.95		0.99	
				601	270						

School (A), Graham; school (B), St. Christopher's; school (C), Leake and Watts; school (X), Children's village; school (Y), St. Joseph's; school (Z), St. John's.

[1] Revised diet (according to Boyd and Drain) with 3 teaspoonfuls of cod liver oil (probably 800 to 1000 international units) per day.
[3] Viosterol expressed at the time in Steenbock units (approximately 3000 international units per day). Institutional diet.
[5] Viosterol expressed at the time in Steenbock units (approximately 6000 international units per day). Institutional diet.
[4] Exposure twice weekly of both back and chest to a quartz mercury lamp. Institutional diet.
[2] Revised diet (according to Boyd and Drain) with no cod liver oil.
[6] One pint of plain reconstituted evaporated milk.
[7] One pint of reconstituted evaporated milk containing 250 international units of natural vitamin D.
[8] One pint of reconstituted evaporated milk containing 400 international units of natural vitamin D.
[9] One pint of reconstituted evaporated milk containing 800 international units of natural vitamin D.
[10] One pint of milk per day in which 400 international units of natural vitamin D had been incorporated at the dairy plant.

DATA CHRONOLOGICALLY ARRANGED (table 1)

1930–1931. In three schools a comparison was made between the effects of the ordinary uncorrected school diet and a diet modelled after that of Boyd, Drain and Nelson ('29) with the difference, however, that 3 teaspoonfuls of cod liver oil were given instead of one. The school diet and the revised diet have been discussed previously (McBeath, '32). Scrupulous supervision with regard to actual intake particularly of the protective foods was carried out by those in charge at table. It will suffice here to say that the school diet was the usual type commonly found in boarding schools with no obvious defects but being short of the optimum allowance of milk, fresh fruits and vegetables. The children were well nourished and healthy according to the current standards. The revised diet supplied an ample allowance of milk (1 quart per day), fresh fruits and vegetables together with a high level of vitamins A and D in the form of cod liver oil (about 800 U.S.P. units of D).

The caries incidence was obviously less in groups receiving the revised diet, thus confirming the findings of Boyd, Drain and Nelson ('29). A direct numerical comparison with these authors is not possible. Boyd and Drain reported principally on the occurrence and arrest of dentinal caries. Our data include enamel caries even in the incipient stages.

1931–1932. During this year the observations of 1930 to 1931 were repeated in the same three schools. As far as possible the groups were reversed so that those which had been 'controls' during the previous year became 'experimental' and vice versa. The data on such reversal of groups are given in table 4.

Only two examinations were made. The results are very similar to those of the previous year.

Two new types of experiments were added. One involved the administration of viosterol and the other the exposure of the skin of chest and back twice weekly to a quartz mercury lamp. The viosterol gave a moderate reduction of caries incidence. The ultraviolet light exposure had an effect com-

parable to that of the feeding of cod liver oil. For details see tables 6 and 7.

1932–1933. During this school year studies with ultraviolet light and viosterol were repeated with results similar to those of the previous year. The viosterol effect again did not equal that of cod liver oil, in spite of the fact that the viosterol dose was doubled.

At school (A) a new type of experiment was introduced, namely the feeding of the Boyd and Drain diet without addition of cod liver oil. This resulted in a distinct but moderate reduction in caries incidence which, by no means, reached the low values recorded when cod liver oil was included.

During this winter the first attempts were made to work with vitamin D concentrates from natural sources. Several lots of concentrates had to be used which turned out to be not of equal potency, and not being used simultaneously in the several groups, the effect, although evident, was very irregular. For this reason the data are omitted from table 1. The following year better control was achieved and larger groups of children were available.

1933–1934. As can be seen from table 1 the work was planned so as to include enough children to yield (combined) groups of about fifty on each phase of the experiment. A batch of carefully assayed vitamin D concentrate, enough to serve for the entire experiment at three levels of vitamin D intake, was incorporated in evaporated milk. These three vitamin D milks and also plain evaporated milk were put up in ½ gallon tins. At each school each day the milk to be used was diluted with equal quantities of water and served to the children in the respective groups in the middle of the morning and in the afternoon. To make the milk consumption least burdensome for those children who were not fond of milk, and to secure the best results in regularity of intake, the milk was slightly flavored with chocolate. Each milk was repeatedly assayed and found to be of the stated potency.

The two 8-ounce glasses consumed contained the daily vitamin D intake given in the tables. In the preliminary paper

(McBeath, '34) this was given in terms of Steenbock units. At the time of introduction of the international standard a conversion factor of 2.7 was suggested on the basis of the average rat reaction in a number of laboratories. A re-check of the milk by means of the international standard showed that our rats at the time were of average healing susceptibility to vitamin D, and that the milk was of the potency stated in table 1.

Table 1 shows that the feeding of the plain evaporated milk gave a slight reduction in caries incidence and that increasing vitamin D intake with the milk gave progressively further reduction. This is shown in more detail in table 5.

Two other groups of children received 1 pint of fresh milk per day in which 400 U.S.P. units of vitamin D had been incorporated in the dairy plant.

TABLE 2

Summary of controls. Experiments in which three examinations were made

	INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH							
	1930–1931		1932–1933		1933–1934		All years	
	Number of cases	Score	Number of cases	Score	Number of cases	Score	Number of cases	Score
Autumn-spring								
(A) Graham	18	2.12	21	2.45	19	2.32	58	2.31
(B) St.Christopher's	11	1.88					11	1.88
(C) Leake and Watts	27	1.80	20	1.66	17	2.64	64	1.98
(Y) St. Joseph's					30	2.73	30	2.73
(Z) St. John's			26	2.92	29	3.09	55	3.04
Totals	56	1.92	67	2.40	95	2.74	218	2.43
Autumn-winter								
(A) Graham	18	2.39	21	2.38	19	1.71	58	2.17
(B) St.Christopher's	11	1.22					11	1.22
(C) Leake and Watts	27	0.93	20	0.77	17	2.35	64	1.26
(Y) St. Joseph's					30	2.92	30	2.92
(Z) St. John's			26	2.79	29	2.45	55	2.61
Totals	56	1.46	67	2.06	95	2.43	218	2.07
Winter-spring								
(A) Graham	18	1.71	21	2.56	19	3.56	58	2.62
(B) St.Christopher's	11	2.87					11	2.87
(C) Leake and Watts	27	3.08	20	2.94	17	3.51	64	3.15
(Y) St. Joseph's					30	2.47	30	2.47
(Z) St. John's			26	3.17	29	5.54	55	3.90
Totals	56	2.60	67	2.91	95	3.51	218	3.09

SUMMARY OF CONTROLS (table 2)

Autumn-winter, winter-spring, autumn-spring periods

The first section of the table compares the first (autumn) examination with the last (spring) examination for each school in each year in which three examinations were made. The two other sections of the table deal with the same observations when these are divided into two sub-periods (autumn-winter and winter-spring). The grand mean score for all schools and all years for the autumn-spring period was 2.43. Considering the fact that neither the diet nor the sunlight exposure was necessarily identical from year to year and school to school variations in the group scores are not surprising.

The results for the autumn-winter and winter-spring periods demonstrated the interesting fact that the caries incidence during the winter-spring period was distinctly higher. The grand total for the autumn-winter period was 2.07, the grand total for the winter-spring period was 3.09. Individual group scores may fall out of line but whenever mean values derived from fifty or more individuals are compared the winter-spring caries incidence is seen to be distinctly higher than that for the autumn-winter period.

The unequal length of periods might be considered open to criticism. It came about largely through the wish to accommodate ourselves to various routine arrangements in the schools. If a period were chosen too short to allow measurable signs of new caries to appear, the score per 100 days would be low. When, therefore, the shorter of the two periods shows the higher score, this cannot be attributed to the shortness of the period.

SUMMER CONTROLS AND CONTROLS UNDER OBSERVATION FOR A YEAR (table 3)

Our understanding of the seasonal effect is considerably extended when we observe those cases which were 'control' during an entire calendar year. Eighty-five individuals have been 'controls' during one school year and were again available the next fall. Seventeen of these came from the 1931–

TABLE 3

Summer controls and controls under observation for a year

	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH				
	Summer	Whole year	Autumn-spring	Autumn-winter	Winter-spring
182 summer controls (including those which were 'experimental' during the previous year)	0.64				
85 controls under observation for a year (omitting the mid-winter examination)	0.57	1.41	2.22		
68 controls under observation for a year (having mid-winter examination) (included in the above)	0.64	1.51	2.32	1.74	3.12

1932 experiments where only two examinations were made. This reduced the recorded autumn-winter and winter-spring entries to sixty-eight. One hundred eighty-two cases, including the 'experimentals' of the previous year, were summer controls with a score of 0.64. This contrasts sharply with the value of 3.12 for the winter-spring period.

TABLE 4

Comparison of experimental and control regime in the same individual

GROUP	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH AUTUMN-SPRING PERIOD		
	Number of cases	Experimental	Control
1930–1931 improved diet + 3 teaspoonfuls cod liver oil	33	0.62	1.46
1931–1932 improved diet + 3 teaspoonfuls cod liver oil	33	0.26	2.25
1933–1934 400 U. vitamin D milk	39	0.63	1.64
All cases	105	0.52	1.75

COMPARISON OF EXPERIMENTAL AND CONTROL REGIME IN THE SAME INDIVIDUAL (table 4)

Table 4 comprises the 105 cases which appeared in the study as controls during 1 year and as experimental subjects during

another. When 'experimental' they were distributed between three groups with daily vitamin D intake as follows:

1930–1931, 3 teaspoonfuls of cod liver oil—33 individuals
1931–1932, 3 teaspoonfuls of cod liver oil—33 individuals
1933–1934, 400 international units of vitamin D in milk—39 individuals

The control observations were made either the year before or the year after the experimental observations.

It will be seen that the same individuals when in control groups in which no added source of vitamin D was given produced a score of 1.75 while with added vitamin D they scored 0.52. Many of the control values date from years and schools where caries incidence was generally low.

TABLE 5

Graded doses of vitamin D given as vitamin D milk

	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH							
	Number of cases		Autumn-spring		Autumn-winter		Winter-spring	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
250 U. vitamin D milk (evaporated)	54	59	1.77	2.91	1.32	2.69	2.47	3.49
400 U. vitamin D milk (evaporated and fluid)	89	36	1.20	2.49	0.77	1.99	1.47	3.64
800 U. vitamin D milk (evaporated)	58	59	0.52	2.91	0.47	2.69	0.56	3.49
Plain evaporated milk	95	95	1.91	2.74	1.34	2.42	3.06	3.55

GRADED DOSES OF VITAMIN D GIVEN AS VITAMIN D MILK (table 5)

These experiments were performed in the winter of 1933–1934 with three different levels of vitamin D intake representing 250, 400 and 800 international units per day. In the case of each group the results were compared with a similarly constituted group which was left on the institutional diet in the same school at the same time. The experiment was so conducted that the several levels of vitamin D intake were distributed in groups of twenty to thirty individuals between four different schools. This resulted in the same individuals serving as controls for the 250 unit and 800 unit levels (see

table 1). Each vitamin D level was represented by at least fifty individuals.

It is clearly seen that there is a gradation of caries incidence with the amount of vitamin D added to the diet. A comparison of the autumn-winter and winter-spring period of study is of particular interest. When we consider the control data it is seen that in each case the winter-spring period gave higher values than the autumn-winter period. In the groups receiving 250 units of vitamin D the autumn-winter period resulted in a score of 1.32 against 2.47 for the winter-spring period, and the mean value for the whole autumn-spring period was reduced only moderately below the value for controls. In the 400 unit groups the reduction below the control value was considerable, but there was still a difference between the autumn-winter and the winter-spring period. This would indicate that 400 units per day is sufficient to keep the caries incidence to low values during the autumn-winter period, but not during the winter-spring period. With 800 units of vitamin D the difference between the autumn-winter and winter-spring period is negligible.

TABLE 6

Irradiated ergosterol (viosterol)

	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH AUTUMN SPRING PERIOD			
	Number of cases		Score	
	Experimental	Control	Experimental	Control
(X) Children's Village (1931–1932)	21	23	1.56	1.93
(Z) St. John's School (1932–1933)	21	26	1.52	2.92
Totals	42	49	1.54	2.45

IRRADIATED ERGOSTEROL (VIOSTEROL) (table 6)

The data on the feeding of viosterol without any further change in the diet reveal a caries inhibiting effect, although not as marked as that produced by exposure of the skin to ultraviolet light with similar food regime.

As with cod liver oil, the possibility of the viosterol not being swallowed was guarded against from the beginning. The nurse required that 'thank you' be clearly enunciated

after the viosterol was received on the tongue and the children remained in the room for some time after.

This topic deserves more study since, as far as we know, a direct comparison of definitely known amounts of viosterol and vitamin D from cod liver oil has not been made.

TABLE 7

Exposure of the skin to ultraviolet light

	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH							
	Number of cases		Autumn-spring		Autumn-winter		Winter-spring	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
(X) Children's Village (1931–1932)[1]	19	23	0.27	1.93				
(Z) St. John's School (1932–1933)	27	26	0.97	2.92	0.88	2.79	1.13	3.17
(Z) St. John's School (1933–1934)	26	29	0.96	3.09	0.95	2.45	0.99	4.54
Totals for three examinations	53	55	0.97	3.01	0.92	2.61	1.06	3.90
Totals for two examinations	72	78	0.78	2.69				

[1] In 1931–1932 only two examinations were made—autumn and spring.

EXPOSURE OF THE SKIN TO ULTRAVIOLET LIGHT (table 7)

To prove beyond question that additional vitamin D brings about certain stated results we should make a change in only this one factor. Natural vitamin D as a chemical individual was not available at the time these studies were carried out. Considerable extra weight can be attached to the evidence for vitamin D being a potent factor in caries prevention by the experiments on exposure of the skin to ultraviolet light.

For the ultraviolet light treatment a quartz mercury lamp was used. The distance from the lamp to the body was 60 inches. Two exposures per week were made on both chest and back. The first exposure was $\frac{1}{4}$ minute on chest and $\frac{1}{4}$ minute on back. Each week the exposure time was increased

by ½ minute, leading to a final exposure of 12 minutes. None of the children experienced any discomfort at any time.

The caries-inhibiting effect of exposure to ultraviolet light as shown in the table is in good agreement with the implications of the summer control data. With some variation from year to year the mean score is 0.78 for seventy-two subjects when the whole autumn-spring period is recorded. The corresponding control value is 2.69 for seventy-eight individuals. Only two groups had the intermediate dental examination. For these the winter-spring period values are probably not significantly higher than those for the autumn-winter period.

DISCUSSION

The results here reported verify the findings of Mellanby and others regarding the effect of vitamin D on incidence of dental caries. On the quantitative side both the optimal dose and the type of vitamin D require further investigation. It would also seem very desirable if a method acceptable to all investigators could be devised and agreed on for the reporting of caries incidence. While the method here employed gives consistent results, there is no reason to believe that in its present form it is the best procedure.

There is no question that the chance for optimal tooth structure and tooth stability is the greatest when an 'ideal' diet is fed. The marked effect of vitamin D, however, together with observations on the effect of season and exposure to ultraviolet light lend much support to the thought that the calcium-phosphorus-vitamin D interrelationship plays a specific role in processes of tooth growth and preservation. This is in no way at variance with the observations that in some species (guinea pig) a vitamin C deficiency can produce marked carious lesions in teeth. Vitamin C and vitamin D are the two factors whose deficiency also produces specific lesions in bones.

When Mrs. Mellanby's first caries studies appeared, immediate ready acceptance was not generally accorded because other theories which excluded nutritional effects were very firmly entrenched. The significance of any measurement of

caries incidence or caries prevention was also an open question. To all corroborative evidence published since that time we can add our observations, which entirely confirm Mrs. Mellanby's judgment on both these points.

Considering all facts adduced so far it seems logical to consider the condition of teeth (re caries) as an indicator of good or faulty nutrition. What particular deficiencies are responsible may vary geographically or with other living conditions. It appears, however, that under most conditions that phase of metabolism which is under the control of vitamin D occupies a very prominent role. This, of course, includes considerations of adequacy of calcium and phosphorus intake.

The question whether, in children, other than nutritional etiologic factors are operative cannot be argued fairly until diets optimal in every respect are devised and these are rigidly maintained for a sufficient length of time and on a sufficient number of individuals to obtain valid results.

The seasonal effect on caries incidence seems to be well established. The marked spontaneous reduction of new lesions during the summer emphasized the extent to which sun exposure without further dietary control affects the picture. This may be held to bear out the analogy between caries and rickets. A recent paper by Mills ('37) in which he indicates a correlation between geographical latitude and caries incidence, also points to available sunshine as playing a definite role. While the experiments with high vitamin D intake and especially the summer controls suggest that vitamin D effects (whether by mouth or through sunlight action) play a prominent role, the rest of the diet, especially the calcium intake, cannot be neglected. It will be of interest to see whether a regime optimal in calcium and vitamin D (or summer sunlight) and also otherwise well balanced will reduce caries incidence to zero. So far no regime has been shown consistently to prevent the occurrence of new carious lesions in a reasonably large group.

It is worthwhile to mention briefly the results of subjecting the data to some of the elementary methods of statistical checking. Besides the mean (M) we have determined the

mean deviation (ε) and the mean deviation of the mean (ε_M) for all the groups or combined groups and have calculated the probability ratio $\frac{Ma - Mb}{\sqrt{\varepsilon_{Ma}^2 + \varepsilon_{Mb}^2}}$ for all the important comparisons which might be made to establish validity. (For definition of terms see Scott ('27). We have convinced ourselves that none of the conclusions drawn from the data need to be questioned on statistical grounds as far as size of groups and calculated deviations about the means are concerned.

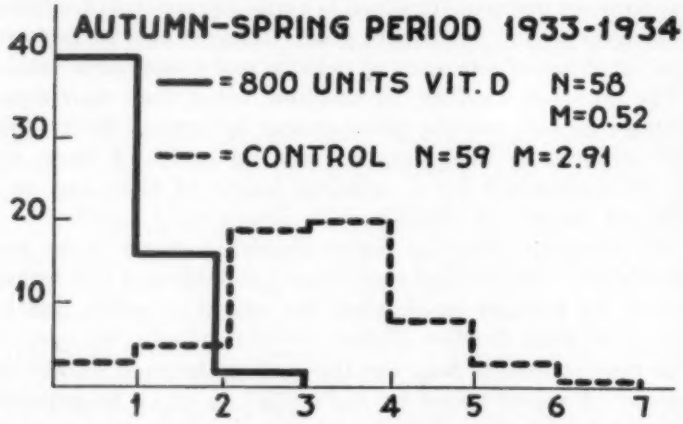

Fig. 1 Frequency polygons to illustrate the distribution of the various degrees of caries incidence in a control group of fifty-nine cases and a group receiving 800 units of vitamin D, fifty-eight cases. The number of new carious surfaces per 100 days is plotted against frequency.

For a comparison of the graded vitamin D milk data with the corresponding control values we find probability ratios ranging from 5 to 12 for all except the comparison of the 250 unit experiment during the winter-spring period, where the probability ratio is 2.2. This makes the last mentioned comparison just significant. The plain evaporated milk groups, which show mean values somewhat better than the controls, give probability ratios of 4 and 5 except during the winter-spring period where the value is below 2. This might be interpreted as meaning that during the height of the caries season the plain evaporated milk is not sufficiently protective

to differ from the controls while at times of less severe caries susceptibility its effect is slight but definite. The probability ratios for comparison of ultraviolet light treatment with its controls are very safe, all being above 6.

As far as the seasonal effect is concerned a comparison between the summer controls and any of the controls for the other periods gives probability ratios of 3 and 4. All values for the comparison of autumn-winter and winter-spring periods in control or experimental groups lead to probability ratios of 3 to 5 with the exception of the high vitamin D intake. In the case of the 800 unit experiments the ratio is 0.6, thus confirming the conclusion previously noted that only with a high vitamin D level of about 800 units will the difference between the autumn-winter and winter-spring periods be obliterated. This applies also to the comparison of the autumn-winter and winter-spring periods of 1930–1931 diet studies when the three schools are combined.

The type of change in the distribution of the number of new carious surfaces brought about in going from a control group to an 800 unit vitamin D group is illustrated in figure 1.

We wish to express our thanks to Dr. William A. Verlin for his very valuable assistance in the dental examinations. Grateful acknowledgment is also due to the Dean Milk Company of Chicago for the very kind gift of evaporated milk and vitamin D evaporated milk. Thanks are also due to the Ille Electric Corporation of New York for the loan and installation of the Burdick solarium lamp.

CONCLUSIONS

1. In a study comprising observations of over 800 children it was found that the incidence of dental caries was seasonal. The greatest incidence was found in late winter and early spring and very low values during the summer.

2. Previous observations on the beneficial effects of vitamin D were verified. The administration of graded amounts of natural (animal source) vitamin D as vitamin D milk

resulted in graded caries prevention. Of the three levels given (250, 400 and 800 international units per day), only the last named was adequate to prevent an increase during the height of the caries season above that of the previous period.

3. Fortifying the diet with 'protective foods' or simply increasing the allowance of milk in the diet led to a moderate reduction in caries when no appreciable vitamin D was added. The change is clear cut in the autumn-winter period but not so definite in the winter-spring period.

4. A reversal of 'control' and 'experimental' regimes during 2 successive years in over 100 cases showed that individual susceptibility to caries was negligible compared to the effect of nutritional factors.

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THE SPECIFIC DYNAMIC EFFECTS OF PROTEINS WHEN ADDED IN DIFFERENT AMOUNTS TO A MAINTENANCE RATION [1]

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ONE FIGURE

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The influence of different levels of intake of protein on the heat production of an animal is of both physiological and nutritional interest. From the latter point of view this question is particularly important, inasmuch as the specific dynamic effect of a food nutrient affects its net energy value. Despite the numerous investigations of this subject on record, the available evidence concerning the dynamic effect of protein when fed in different quantities is not incontrovertible.

Rubner ('02) made the observation that the dynamic effect of a protein is proportional to the amount ingested. This was found to be essentially true by Williams, Riche and Lusk ('12), when feeding to a dog large quantities of meat.

On the contrary, in a series of experiments in which a man was fed casein exclusively, in varying quantities, Gigon ('11) found a progressive increase in the specific dynamic value per unit of the protein ingested, from the lowest to the highest level. Similarly, Weiss and Rapport ('24) observed that when increasing quantities of beef were given to a dog, the increase in heat production was not a linear function of the food ingested. This increase was found, however, to be, in general, directly proportional to the amount of protein metabolized.

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In all of the foregoing investigations the dynamic effect of protein was determined as the increase in heat production over the post-absorptive metabolism.

In the present study the problem was approached from a somewhat different angle, in accord with the following conception.

In a recent publication by Kriss, Forbes and Miller ('34) evidence was presented to show that the sparing action of food nutrients upon body nutrients, which takes place below maintenance, is likely to cause confusion of the apparent dynamic effects of these nutrients, and that, from the point of view of nutrition, more significant measurements of specific dynamic effects of food nutrients could be made if the heat production of an animal maintained in energy and nitrogen equilibrium, instead of the fasting metabolism, were used as the base value.

Consequently, in the experiments recorded herein, the main object was the determination of the specific dynamic effects of different proteins, as affected by the quantity fed, when these nutrients were given as supplements to a diet providing the requirements of energy and nitrogen equilibrium.

EXPERIMENTAL

The subjects of this investigation were twenty-four male rats, approaching maturity, and weighing approximately 200 gm. each. When this weight was reached the rats were removed from the stock colony and placed in individual cages. They were then fed 8.0 gm. per day of a basal ration composed of 93.7% of calf meal (described by Forbes, Kriss and Miller, '34) and 6.3% of butterfat. This diet maintained the rats at constant weight, and was found in a previous study (Kriss and Voris, '37) to be adequate to maintain rats of this size in approximate energy and nitrogen equilibrium.

The rats were divided into three groups of eight individuals, each group receiving a different protein food as the test substance. Group 1 received casein; group 2 received gelatin, while group 3 received heart muscle. Each of these substances was fed as a supplement to the basal ration in quanti-

ties of 1.5 gm. and 3.0 gm. All rats were subjected, at approximately weekly intervals, to respiration experiments while on each of the following daily dietary treatments, and in the order indicated: 1) fasting, 2) 8.0 gm. basal ration, 3) 8.0 gm. basal ration plus 1.5 gm. protein supplement, 4) 8.0 gm. basal ration, 5) 8.0 gm. basal ration plus 3.0 gm. protein supplement, 6) 8.0 gm. basal ration.

The particular substances used as the protein supplements, in the supermaintenance periods, were selected primarily because of the wide differences in their amino acid composition.

The chemical composition of the basal ration and of the protein supplements is given in table 1. The casein, labeled

TABLE 1

Composition of basal ration and protein supplements

MATERIAL	MOISTURE	NITROGEN	ETHER EXTRACT	ASH	ENERGY
	%	%	%	%	*calories per gram*
Basal ration	8.99	3.00	9.25	5.21	4358
Casein	9.15	12.99	0.22	0.92	5175
Basal ration	9.40	3.29	9.62	4.90	4362
Nutrient gelatin	8.17	15.22	0.13	3.48	4573
Basal ration	6.95	3.34	10.28	5.07	4447
Heart muscle	1.64	12.95	9.32	5.02	5559

'pure,' was obtained from Pfanstiehl. The gelatin was of the 'Barto Nutrient' grade, dehydrated. The heart muscle, as fed, was prepared from beef heart by removal of visible fat, freezing, drying in a Hempel desiccator, and grinding to a fine powder.

As stated above, the protein supplements were given in only two different quantities. The larger quantity (3.0 gm.) closely represented the maximum which all groups of rats would clean up, in addition to the basal ration, in the reasonably short time required by the method of experimentation. No smaller quantity than 1.5 gm. was tried, since significantly smaller quantities could not be expected to yield consistent heat increment values.

The proportions of protein (N × 6.25) in the various rations, in relation to the total quantity of dry matter, were, therefore, as follows: for group 1—basal ration, 20.6%, basal ration plus 1.5 gm. casein, 31.4%, basal ration plus 3.0 gm. casein, 39.3%; for group 2—basal ration, 22.7%, basal ration plus 1.5 gm. gelatin, 35.6%, basal ration plus 3.0 gm. gelatin, 45.0%; for group 3—basal ration, 22.4%, basal ration plus 1.5 gm. heart muscle, 32.3%, basal ration plus 3.0 gm. heart muscle, 39.4% protein.

The metabolizability of the basal ration and of the protein supplements, at the two levels, was determined in a separate series of experiments, the results of which have been published elsewhere (Kriss and Voris, '37).

The practice of giving the daily allowance of food in two equal portions was again followed. This method results in an approximately uniform rate of metabolism, the respiration measurements, carried on during several hours of the day, therefore, being satisfactorily representative of the entire 24 hours.

All respiration measurements were made in periods of 6 to 7 hours.

The fasting experiments began 24 hours after food, following a period of feeding on the basal ration of not less than a week. The determination of the fasting metabolism served as a basis for correcting the heat production of the subjects in all experimental periods to a basis of uniform body weight. Immediately following the measurements of the fasting metabolism the rats were placed back on the basal ration.

The respiratory metabolism representing the various rations was determined at the end of approximately 1 week's feeding on the ration to be tested. These measurements began in each case soon after the morning meal was consumed.

The weekly intervals were chosen in consideration of the uniformity and consistent character of the metabolic results obtained under similar conditions by Kriss, Forbes and Miller ('34), and in the light of subsequent work by Kriss and Voris (unpublished results) which showed that if rats are kept on

a constant supermaintenance diet for relatively long periods of time (2 weeks or longer) the dynamic effect of the diet may be obscured by secondary changes in the metabolism, resulting from the changes in age and in body weight.

As a check on the possible effect of age and body weight on the metabolism during the weekly periods, each supermaintenance period was preceded and followed by a period of maintenance feeding.

The schedule of respiration experiments and the body weights are presented in table 2.

The determinations of the respiratory exchange were carried out by means of the apparatus which was previously described by Forbes, Kriss and Miller ('34), but which has since undergone certain modifications. These included 1) the substitution of a constant temperature air-bath for the constant temperature water-bath, 2) provision for the visibility of the rats by the use of reflecting mirrors, and 3) the installation of a device for recording the activity of the subjects while in the respiration chamber. The latter device, however, was not installed until after the experiments with the casein group had been completed.

The activity recorder (fig. 1) consists of a 'work adder'[2] which is placed above the chamber and connected to it by means of a metal rod, the chamber (glass jar) being suspended by springs. Any vertical movement of the chamber is registered on the graduated disc of the work adder, while complete revolutions of this disc are registered on an improvised scale.

Readings of activity were recorded at hourly intervals corresponding to the measurements of the CO_2 production. These records aided somewhat in the interpretation of the hourly CO_2 measurements, and in the elimination of certain data, obviously affected by activity, from the average results.

The computations of the heat production were made in accord with the general procedure followed previously by Kriss, Forbes and Miller ('34), while the protein metabolism

[2] This was purchased from the Harvard Apparatus Co., Boston, Massachusetts.

TABLE 2

Schedule of respiration experiments and body weights

RAT NO.	1 FASTING		2 BASAL RATION 8.0 GM. DAILY		BASAL RATION PLUS 1.5 GM. CASEIN		BASAL RATION 8.0 GM. DAILY		BASAL RATION PLUS 3.0 GM. CASEIN		BASAL RATION 8.0 GM. DAILY	
	Date	Body weight	Date	Body weight	Date	Body weight	Date	Body weight	Date	Body weight	Date	Body weight
	1936	*gm.*	*1936*	*gm.*	*1936*	*gm.*	*1936*	*gm.*	*1936*	*gm.*	*1936*	*gm.*
11	May 5	201	May 12	209	May 20	221	May 26	213	June 2	238	June 9	224
12	May 5	207	May 12	217	May 20	233	May 26	225	June 2	247	June 9	235
13	May 6	205	May 13	214	May 21	224	May 27	224	June 3	247	June 10	236
14	May 6	193	May 13	204	May 21	211	May 27	211	June 3	240	June 10	230
15	May 7	198	May 14	201	May 22	214	May 28	213	June 4	233	June 11	225
16	May 7	203	May 14	210	May 22	215	May 28	215	June 4	235	June 11	228
17	May 8	213	May 15	219	May 23	228	May 29	225	June 5	242	June 12	233
18	May 8	208	May 15	210	May 23	220	May 29	220	June 5	238	June 12	233
					BASAL RATION PLUS 1.5 GM. GELATIN				BASAL RATION PLUS 3.0 GM. GELATIN			
21	Nov. 9	171	Nov. 16	167	Nov. 23	181	Dec. 1	176	Dec. 10	196	Dec. 17	187
22	Nov. 9	187	Nov. 16	186	Nov. 23	198	Dec. 1	189	Dec. 10	212	Dec. 17	205
23	Nov. 10	191	Nov. 17	193	Nov. 24	208	Dec. 2	198	Dec. 11	222	Dec. 18	216
24	Nov. 10	178	Nov. 17	183	Nov. 24	193	Dec. 2	187	Dec. 11	206	Dec. 18	202
25	Nov. 11	169	Nov. 18	174	Nov. 25	182	Dec. 3	183	Dec. 14	207	Dec. 21	206
26	Nov. 11	167	Nov. 18	174	Nov. 25	188	Dec. 3	185	Dec. 15	204	Dec. 21	204
27	Nov. 12	165	Nov. 19	172	Nov. 27	185	Dec. 4	181	Dec. 15	188	Dec. 22	188
28	Nov. 12	176	Nov. 19	171	Nov. 27	179	Dec. 4	172	Dec. 14	191	Dec. 22	183
					BASAL RATION PLUS 1.5 GM. HEART MUSCLE				BASAL RATION PLUS 3.0 GM. HEART MUSCLE			
	1937		*1937*		*1937*		*1937*		*1937*			
31	March 2	188	March 8	197	March 15	208	Mar. 22	206	Mar. 30	225	Apr. 5	226
32	March 2	187	March 8	198	March 15	209	Mar. 22	204	Mar. 30	226	Apr. 5	224
33	March 3	186	March 9	188	March 16	198	Mar. 23	198	Mar. 31	221	Apr. 6	215
34	March 3	187	March 9	191	March 16	203	Mar. 23	207	Mar. 31	234	Apr. 6	226
35	March 4	166	March 10	169	March 18	186	Mar. 24	183	Apr. 1	207	Apr. 7	202
36	March 4	192	March 10	191	March 18	211	Mar. 24	207	Apr. 1	229	Apr. 7	223
37	March 5	186	March 11	178	March 19	203	Mar. 25	206	Apr. 2	225	Apr. 8	222
38	March 5	177	March 11	179	March 19	197	Mar. 25	197	Apr. 2	224	Apr. 8	218

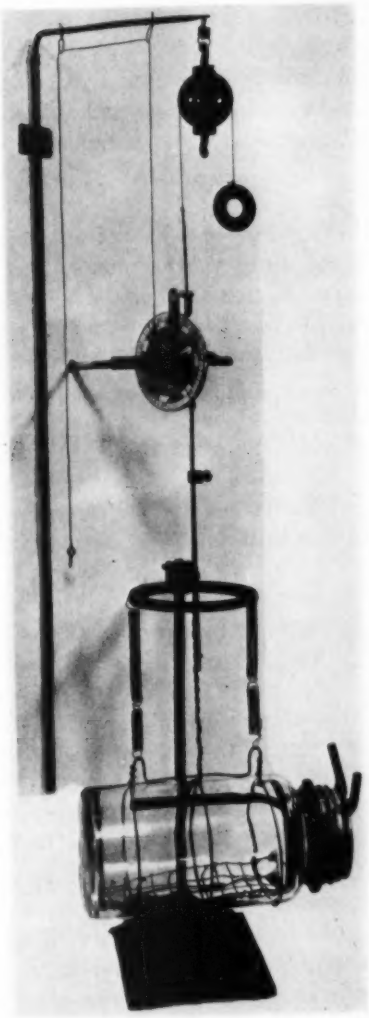

Fig. 1 Work adder connected with the respiration chamber.

was computed on the basis of the results obtained by Kriss and Voris ('37).

In order to provide a basis for calculating the heat production per unit of empty body weight, with each dietary treatment, the fill was determined in control animals which were of approximately the same age and weight as the experimental subjects.

RESULTS

The average hourly heat production of the individual rats, under the different dietary treatments, was computed, for comparative purposes, per 200 gm. of empty body weight. These data, as well as the heat increments resulting from the addition of the protein supplements to the basal ration, are set forth in table 3.

The heat production representing the basal ration exhibits a fair degree of uniformity among the different groups of animals, as well as among the animals of each group. The average results of each group show only a slight decline in metabolism from the initial to the final maintenance period, this decline being relatively greatest with the casein group. No significant difference is observed in the metabolism of the other two groups between the intermediate and final maintenance periods. Contributing to the uniformity of these results is the fact that no extensive changes in body weight occurred during the rather short experimental feeding periods.

No less consistent than the foregoing results are the data for heat production in the supermaintenance periods. In each case the addition of the protein supplement to the basal ration resulted in an increased metabolism, and, with only one exception (rat 21), the larger quantity of protein caused the greater heat increment.

The hourly increases in heat production of the individual rats, due to the supplements, are presented in the last two columns. In the computation of these heat increments the average heat production for two maintenance periods (one immediately preceding and one immediately following the supermaintenance period) was used as the base value. This

TABLE 3

Hourly heat production of rats per 200 gm. of empty body weight as influenced by addition of protein supplements to a basal maintenance ration

RAT NO.	BASAL RATION 8.0 GM. DAILY			BASAL RATION PLUS PROTEIN SUPPLEMENT		INCREASE IN HEAT PRODUCTION DUE TO SUPPLEMENT	
	Initial period	Intermediate period	Final period	1.5 gm. casein	3.0 gm. casein	1.5 gm. casein	3.0 gm. casein
	calories	*calories*	*calories*	*calories*	*calories*	*calories*	*calories*
11	840	830	792	975	1134	140	323
12	800	814	799	933	1078	126	271
13	891	820	789	934	1156	78	351
14	853	808	815	943	1112	112	300
15	877	842	809	989	1093	129	267
16	775	781	774	907	990	129	212
17	812	800	733	928	1007	122	240
18	851	826	788	967	1063	128	256
Average	837	815	787	947	1079	121	278
Standard	36.8	17.8	23.8	25.7	54.3	17.7	42.3
deviation	±6.21	±3.00	±4.01	±4.33	±9.16	±2.98	±7.13
				1.5 gm. gelatin	3.0 gm. gelatin	1.5 gm. gelatin	3.0 gm. gelatin
21	838	903	839	975	973	104	102
22	846	879	821	936	1020	73	170
23	757	748	801	868	955	115	180
24	808	777	831	858	946	65	142
25	872	858	846	1006	1045	141	193
26	856	838	830	947	988	100	154
27	868	786	840	915	947	88	134
28	928	833	840	956	1002	75	165
Average	847	828	831	933	985	95	155
Standard	46.7	50.0	13.5	47.4	33.8	23.6	26.9
deviation	±7.87	±8.43	±2.28	±7.99	±5.70	±3.98	±4.53
				1.5 gm. heart muscle	3.0 gm. heart muscle	1.5 gm. heart muscle	3.0 gm. heart muscle
31	899	917	884	981	997	73	96
32	839	836	830	895	961	57	128
33	839	818	807	902	976	73	163
34	841	852	801	903	992	56	165
35	965	849	860	996	1011	89	156
36	871	880	850	994	1021	118	156
37	904	819	869	945	1010	83	166
38	853	837	896	947	1035	102	168
Average	876	851	850	945	1000	81	150
Standard	41.5	31.1	32.4	39.4	22.5	20.0	23.6
deviation	±7.00	±5.24	±5.46	±6.64	±3.79	±3.37	±3.98

procedure is believed to compensate for any slight changes in the rate of metabolism incidental to the changes in age and body weight.

It will be observed that the heat increments of the larger quantities of protein supplements are more consistent than those obtained for the smaller quantities. This is to be expected. In general, heat increments are subject to relatively large experimental errors, because their determination necessarily involves a comparison of results obtained in different experimental periods.

The increases in heat production caused by 3.0 gm. of casein are, with one exception (rat 13), approximately twice as great as those caused by 1.5 gm. casein. The average values for the entire group of eight animals are 121 calories per hour for the smaller quantity of casein, and 278 calories for the larger quantity of casein. If the aberrant results of rat 13 are omitted, the averages are 127 calories and 267 calories, respectively, these values being closely proportional to the quantities of protein supplement.

Gelatin in the amount of 1.5 gm. caused an average heat increment of 95 calories per hour as compared with the increase of 155 calories caused by 3.0 gm. of gelatin. The results with rat 21 present the only case in which the heat increment of 3.0 gm. of protein supplement was found not to be larger than the increment for 1.5 gm. protein supplement.

The ingestion of 1.5 gm. of heart muscle resulted in an average increase in heat production of 81 calories per hour, while 3.0 gm. of the same substance caused an increase of 150 calories per hour. These results also show an approximate proportionality between the quantity of food ingested and the dynamic effect.

In table 4 are presented the average results of the calculations of the specific dynamic effects and of the net energy values of the protein supplements at the two levels of feeding.

The values for metabolizable energy of the supplements represent the following percentages of the gross energy: for 1.5 gm. casein, 81.4%, for 3.0 gm. casein, 82.9%; for 1.5 gm.

TABLE 4

Specific dynamic effects and net energy values of casein, gelatin and heart muscle at two different levels of feeding

DAILY SUPPLEMENT	GROSS ENERGY	METABOLIZABLE ENERGY	TOTAL NITROGEN	NITROGEN RETAINED IN THE BODY	PROTEIN KATABOLIZED		SPECIFIC DYNAMIC EFFECTS				NET ENERGY PER GRAM	
					Urinary nitrogen		Per 24 hours	Per 100 cals. of protein katabolized	Per cent of metabolizable energy			
	calories	*calories*	*mg.*	%	*mg.*	*calories*	*calories*	*calories*			*calories*	*calories*
1.5 gm. casein	7,763	6,319	195	6.0	176	5504	2904	52.8	46.0	48.3 [1]	2277	2181 [1]
3.0 gm. casein	15,525	12,870	390	17.2	310	9619	6672	69.4	51.8	49.8 [1]	2066	2154 [1]
1.5 gm. gelatin	6,860	5,598	228	13.9	188	4520	2280	50.4	40.7	38.2 [2]	2212	2308 [2]
3.0 gm. gelatin	13,719	11,044	457	21.5	347	7925	3792	47.8	34.3	35.4 [2]	2417	2377 [2]
1.5 gm. heart muscle	8,339	7,372	194	29.1	130	3578	1944	54.3	26.4		3619	
3.0 gm. heart muscle	16,677	14,159	389	28.6	260	6734	3600	53.5	25.4		3523	

[1] Omitting results of rat 13.
[2] Omitting results of rat 25.
[3] Omitting results of rat 21.

gelatin, 81.6%, for 3.0 gm. gelatin, 80.5%; for 1.5 gm. heart muscle, 88.4%, for 3.0 gm. heart muscle, 84.9%. These percentages are as determined by Kriss and Voris ('37).

The data representing protein katabolism were derived on the following basis: of the nitrogen ingested in the form of the supplements, the following percentages were found by Kriss and Voris ('37) to be excreted in the urine: for 1.5 gm. casein, 90.5%, for 3.0 gm. casein, 79.6%; for 1.5 gm. gelatin, 82.6%, for 3.0 gm. gelatin, 75.9%; for 1.5 gm. heart muscle, 67.0%, for 3.0 gm. heart muscle, 66.9%. The calorific values per milligram of urinary nitrogen were as follows: for 1.5 gm. casein, 31.27 calories, for 3.0 gm. casein, 31.03 calories; for 1.5 gm. gelatin, 24.04 calories, for 3.0 gm. gelatin, 22.84 calories; for 1.5 gm. heart muscle, 27.52 calories, for 3.0 gm. heart muscle, 25.90 calories.

The dynamic effects of the protein supplements are expressed 1) in calories per 24 hours, 2) in calories per 100 calories of extra protein katabolized, and 3) as per cent of the metabolizable energy of the supplements.

On all three bases the average results for all the animals used in these experiments are given. In some cases, as indicated, the average results for seven animals (omitting certain extreme results) are also included. This was done in order to bring out more clearly the significance of the data.

The results show that whether the specific dynamic effects of the proteins are expressed as per cent of extra protein katabolized, or as per cent of the total metabolizable energy of the protein supplements, no significant differences in the specific dynamic values resulted from the feeding of different quantities of the proteins.

The specific dynamic effect of casein, expressed as per cent of the metabolizable energy, is somewhat less at the lower level of feeding (46.0%) than that obtained at the higher level (51.8%). This difference, however, largely disappears when the apparently aberrant results of rat 13 are omitted from the average (compare 48.3% and 49.8%).

These values for the specific dynamic effect of casein are appreciably higher than the value (31.4%) obtained in a previous experiment by Kriss, Forbes and Miller ('34), when 2 gm. of casein were added to a basal maintenance ration. In the investigation just cited, however, the subjects (rats) were much younger than in the present one. It is possible, therefore, that the difference between the specific dynamic values of casein as determined in the two experiments is at least partly due to the influence of age on the utilization of the nutrient.

When expressed per 100 calories of extra protein katabolized, the specific dynamic effect of casein (average for all rats) is considerably greater at the higher level (69.4 calories) than at the lower level (52.8 calories). If the results of rat 13 be omitted, the average dynamic values would be 55.5 calories and 66.6 calories, per 100 calories of the protein katabolized at the lower and the higher levels, respectively. Although this difference is of appreciable magnitude it can hardly be considered significant in view of the results obtained with the other protein supplements.

Gelatin, on the contrary, shows a somewhat higher specific dynamic effect at the lower level than at the higher level of feeding. The differences are, however, not large. The average specific dynamic effect per 100 calories of extra protein katabolized, for all animals, is 50.4 calories at the lower level, as compared with 47.8 calories at the higher level. The corresponding averages for seven rats are 47.3 and 49.3 calories, respectively. Expressed as per cent of the metabolizable energy, the average specific dynamic effect for the seven animals is 38.2%, at the lower level, as compared with 35.4% at the higher level.

The specific dynamic effect of the heart muscle, expressed either as per cent of the metabolizable energy, or per 100 calories of protein katabolized, is almost identical at the two planes of nutrition. On the basis of metabolizable energy the dynamic values at the lower and the higher levels of feeding are 26.4 and 25.4%, respectively. The dynamic values per

100 calories of extra protein katabolized, at the two stated planes are 54.3 and 53.5 calories, respectively.

It will be observed that while the specific dynamic values, expressed as per cent of metabolizable energy, show considerable differences among the different proteins tested, these differences largely disappear when the specific dynamic effects are expressed in relation to the calories of protein katabolized. In other words, a closer correlation is revealed between the dynamic effects of the various proteins fed and the resulting increases in protein katabolism than there is between the dynamic effects of the proteins and their total metabolizable energy (including body gain). It is possible, however, that these relationships were somewhat affected by the different fat content of the protein supplements (see table 1).

Of the three protein supplements studied casein shows the greatest and heart muscle shows the smallest specific dynamic effect when this is expressed as per cent of the metabolizable energy. The heart muscle as used contained an appreciable amount (9.32%) of lipid material, but its nitrogen content was identical with that of the casein. However, the total increases in heat production caused by the heart muscle at both levels (1944 and 3600 calories) are considerably smaller than the corresponding heat increments of casein (2904 and 6672 calories). It is clear, therefore, that the comparatively low dynamic value obtained for heart muscle, when expressed as a percentage of the metabolizable energy, may be due only in part to its fat content. Another factor which appears to be prominently associated with this low dynamic value is the relatively greater retention of nitrogen in the body (and smaller urinary nitrogen excretion) caused by the heart muscle preparation.

The data for nitrogen retention given in table 4 are based on the previous experiments by Kriss and Voris ('37). Of the total nitrogen ingested as heart muscle 29.1% and 28.6%, were found to be retained in the body at the low and the high levels, respectively, as compared with 13.9 and 21.5% for gelatin, and 6.0 and 17.2%, for casein. The greatest utilization of nitrogen of heart muscle is expected in view of the

relatively complete nature of this protein as compared with the other two. The somewhat greater deposit of nitrogen caused by gelatin, as compared with casein, was not expected, but was apparently effected through the combination with the protein of the basal ration. The relatively high specific dynamic effect of casein, expressed as per cent of the metabolizable energy, is correlated, therefore, with the smallest retention of nitrogen in the body.

The inverse relationship observed between the nitrogen retention in the body, caused by the protein supplements, and their specific dynamic effects, expressed as percentages of their metabolizable energy, together with the relatively closer correlation shown between the increases in heat production and the increases in protein katabolism, lend support to the belief held by Rubner ('02) that protein deposited exerts little, if any, specific dynamic effect.

On the other hand, the results representing the dynamic effects of the protein supplements in relation to the protein katabolized are quite in accord with the findings of Rapport ('24) that casein, gelatin and beef protein have approximately the same specific dynamic action. Obviously, much depends on the way the specific dynamic effects are expressed. In the experiments of Rapport, amounts of the various protein materials with equal content of nitrogen were fed to a fasting dog, and the specific dynamic effects were expressed as per cent of the basal metabolism. There is therefore no basis for quantitative comparisons of our results with those of Rapport.

From the point of view of the present study the most important observation is that, however the results be expressed, the plane of protein intake shows hardly any significant influence on the specific dynamic values of the nutrients, the total protein content of the protein-supplemented rations varying from 31.4 to 45.0%.

In the last two columns of table 4 are presented the net energy values of the protein supplements. In the last column are given the average results for seven rats, while the column preceding the last one contains the averages for all eight rats.

The net energy of the supplements was calculated, in the usual manner, by subtracting from the metabolizable energy of the daily supplement the calories representing the dynamic effect per 24 hours. The results are expressed in calories per gram.

The net energy values of the supplements are, as expected, in inverse relation to their specific dynamic effects, casein having the lowest and heart muscle having the highest net energy value. The comparatively high net energy value of the heart muscle is undoubtedly also due in part to the relatively greater metabolizability of this substance which in turn may be partly attributed to its lipid content.

All three protein supplements show close agreement between their respective net energy values determined at the two planes of nutrition.

The results indicate that within a certain range above the maintenance plane of nutrition the specific dynamic effects and net energy values of proteins are not significantly affected by the quantity of the protein fed, and that the heat production of energy and nitrogen equilibrium may be used satisfactorily as a base value.

Further investigation is necessary to determine definitely whether the same principle applies to other nutrients and to mixed rations.

SUMMARY AND CONCLUSIONS

The specific dynamic effects of dried heart muscle, casein and gelatin were determined with rats when each of these protein materials was given as a supplement to a basal maintenance ration in quantities of 1.5 gm. and 3.0 gm. per day, the heat production of the animals while on the maintenance ration being used as the base value.

Of the three protein supplements tested casein showed the greatest and heart muscle showed the smallest dynamic effect when this was expressed either as total calories or as a percentage of the metabolizable energy. Accordingly, the net energy value of heart muscle was the greatest and that of casein was the smallest.

An inverse relation was observed between the nitrogen retention in the body, caused by the protein supplements, and their dynamic effects expressed as percentages of their metabolizable energy. The results support Rubner's belief that deposited protein has little, if any, specific dynamic effect.

No significant differences were observed between the specific dynamic values of the proteins when fed in the different quantities.

The net energy values of the protein supplements were practically identical at the two different planes of feeding.

The results indicate that the specific dynamic effects of proteins may be satisfactorily determined within a certain range above the maintenance plane of nutrition with the heat production of energy and nitrogen equilibrium as the base value.

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THE EFFECT OF FORCED AIR CURRENTS AND CLOTHING ON RADIATION AND CONVECTION [1]

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THREE FIGURES

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The technic of separating radiation from conduction has been described by Hardy and Du Bois ('38 a); and the results of the basal experiments have been published in the previous paper ('38 b). Practically all of the basal hours were followed immediately by some superimposed factor such as exercise or an electric fan and the resulting changes in heat production and heat loss compared with the basal control.

Barr and Du Bois ('18) after studying malarial chills in the Sage calorimeter asked the following questions.

> First, how is it possible that two individuals of the same size can eliminate the same amount of heat if one has a cool skin and the other a warm skin? Second, how can one explain the fact that the heat elimination of a malarial patient remains almost exactly the same per hour during the period before the chill, during the period of chill and during the period of high continuous temperature immediately after the chill?

They themselves explained this by means of a diagram showing with a warm skin the greatest cooling of the blood near the skin. In the case of the cool skin they indicated that the greatest cooling of blood took place at a distance of perhaps 2 to 3 cm. below the skin. Their explanation has been questioned by several writers and their statement of facts has been doubted since it apparently conflicted with Newton's law of cooling.

[1] Clinical Calorimetry, no. 51.

This paradox puzzled us for many years and finally we realized that it could be solved only by a quantitative study of the factors involved in heat loss under various conditions.

The most striking factor studied was the effect of air movement. A large electric oscillating fan was placed at the end of the calorimeter in the corner next to the subject's right foot. The oscillations, at the rate of 9 per minute, blew the air first along the man's right side and then diagonally across his body. The velocity of the air movement over the skin surface could not be measured because of the great amount of turbulence caused by the repeated reflection of the air stream from the walls and sides of the box. At the lowest temperature, 27.4°C., the fan which was producing 16.13 cal. per hour made the skin uncomfortably cool for the first 10 minutes and then gradually became less uncomfortable. At 29°C. there was no discomfort even though the fan was going much faster, producing 36.5 cal. per hour. In the experiments at 31.7°C. and 32.2°C. there was slight sweating in the axillae but no discomfort. At 34.0°C. and 34.7°C there was slight sweating over the upper parts of the bodies and the least extra exertion, such as taking surface temperature measurements, produced generalized sweating. The fan at these temperatures made the men feel a little more comfortable or rather a little less uncomfortable. It must be remembered that the men were naked and exposed to humidities of 25 to 50%.

The first surface temperature measurements were made while undressing, the second in the preliminary period, 10 to 15 minutes before the start of the respiration experiment. The first hour was basal with no fan or temperature measurements. In the second or transition period of 30 to 45 minutes, the surface temperature was measured in twenty places, then the fan was started and the surface measured again. In the third period, 1 hour in length, the surface was measured once or twice. The last measurements were made after the end of the third period. The fan was still running. The data for six experiments on the two subjects are given in table 1.

TABLE 1
Experimental data on experiments with electric fan

TABLE 1

Experimental data on experiments with electric fan

SUBJECT, DATE, WEIGHT, REMARKS	END OF PERIOD	CO_2	O_2	RQ	H_2O	URINARY N. PER HOUR	PULSE RATE	TOTAL HEAT PRODUCED	CALORIMETER TEMPERATURE	CALORIMETER HUMIDITY	RECTAL TEMPERATURE	TOTAL HEAT ELIMINATED	SKIN TEMPERATURE		RADIATION	CONVECTION	VAPORIZATION
													Time	Temperature			
EFDB. March 13, 1935, 74.7 kg.	11.15	gm.	gm.		gm.			Cal.		%	37.25	Cal.	10.56	32.8	%	%	%
1st period basal; 2nd period	12.15	23.2	20.7	0.82	35.4	0.56	54	68.7	27.38	25	37.10	77.4	11.18	33.0	58	15	27
quiet with electric fan running	1.15	25.9	24.2	0.78	39.5	0.63	50	79.7	27.48	25	37.03	99.2	12.18	32.8	40	37	23
													12.42	32.2			
													1.02	32.0			
													1.18	32.2			
EFDB. May 15, 1935, 77.3 kg.	11.07										37.26		10.50	33.9			
1st period basal; 2nd period	12.07	22.6	16.2	1.01	36.7	0.47	55	66.4*	28.82	24	37.22	71.2	12.12	33.7	59	11	30
moving, electric fan; 3rd	12.43	17.2	18.3	0.68	24.7	0.44	52	59.5	29.05	24	37.15	58.6	12.35	33.6	42	31	27
period quiet, electric fan.	1.43	23.6	20.5	0.84	36.4		49	68.5	29.40	24	37.15	80.6	1.15	32.9	41	33	26
* Calc. from CO_2													147	33.3			
EFDB. May 22, 1937, 76.8 kg.	10.41										37.20		10.27	34.0			
1st period basal; 2nd period	11.41	23.7	20.6	0.84	38.9		56	68.9	30.42	29	37.14	72.4	11.45	34.4	44	25	31
moving, fan on; 3rd period	12.16	16.4	13.5	0.89	26.4	0.57	53	45.6	30.54	29	37.17	49.7	12.07	34.0	36	33	31
quiet, fan on	1.16	25.6	22.4	0.83	40.4	0.56	53	74.8	30.59	29	37.19	82.8	12.43	33.7	31	40	29
													1.03	33.7			
JDH. March 4, 1937, 67.1 kg.	10.44										37.00		10.27	34.9			
1st period basal; 2nd period,	11.44	20.5	18.8	0.79	44.9		66	62.5	31.47	28	36.98	63.8	12.00	34.8	39	20	41
fan started; 3rd period quiet,	12.32	19.5	15.1	0.94	43.3		64	52.1	31.67	27	37.02	55.3	12.20	34.3	30	24	46
fan going	1.32	23.9	21.0	0.83	60.7	0.34	64	70.4	31.77	29	37.03	76.3	12.37	34.4	24	28	46
													1.48	34.1			
EFDB. May 29, 1935, 76.3 kg.	10.55										37.44		10.23	34.9			
1st period basal; 2nd period	11.55	24.0	19.7	0.89	60.0		57	66.9	32.20	34	37.33	69.4	12.00	35.2	35	12	51
moving, fan started; 3rd	12.35	17.1	13.9	0.89	53.8		55	47.5	32.25	34	37.35	56.0	12.20	34.8	26	18	56
period quiet, fan going	1.35	24.3	20.7	0.86	68.7	0.38	55	69.8	32.28	32	37.29	81.9	12.40	34.7	27	23	50
													1.40	35.1			
JDH. March 9, 1936, 67.3 kg.	11.02										36.96		10.42	35.4			
1st period basal; 2nd period	12.02	21.1	17.2	0.90	82.7		66	58.4	33.98	40	36.97	67.3	12.15	35.1	14	14	72
quiet, fan started; 3rd period,	12.44	16.6	14.0	0.86	81.0		64	47.3	33.95	42	36.98	54.1	12.27	34.8	9	4	87
restless, fan going	1.44	22.3	18.9	0.86	99.3	0.33	66	63.7	34.00	45	37.02	70.6	12.55	34.8	9	9	82
													1.55	34.9			
EFDB. June 3, 1935, 76.0 kg.	11.02										37.41		10.36	35.4			
1st period basal; 2nd period	12.02	24.1	20.0	0.88	110.2		59	67.3	34.73	44	37.40	73.5	12.06	35.2	5	7	88
quiet, fan started; 3rd period	12.37	15.9	12.6	0.91	71.1		57	42.9	34.62	46	37.33	47.1	12.23	35.0	4	5	91
quiet, fan going	1.37	24.4	20.8	0.85	124.0	0.40	58	69.5	34.63	49	37.42	80.0	12.40	35.3	4	5	91
													1.06	35.5			
													1.38	35.5			

The experiments are arranged in order of increasing calorimeter temperature. The first basal periods have been considered in detail and are included here as control periods for the fan experiments. In all except the first experiment a transitional period of about 35 minutes was interposed between the basal hour and the fan hour. This was to allow the fan to be started and the calorimeter to come into gaseous and thermal equilibrium. The rises in heat production seen during the first fan periods are artifacts and are due to blowing off of adsorbed gases on the calorimeter walls. As soon as equilibrium is re-established with the fan running the measurement of heat production is again valid, and the fan is kept running, once it is started, until the subject is removed from the calorimeter. The transition periods are included in order to follow the immediate transitory effects of the fan.

The data are summarized in figure 1 and show that the fan had little or no effect in changing the total metabolism. The moderate increases of 2 to 10 calories per hour in the third period could be accounted for by the slight exertion necessary to make the surface measurements. The rectal temperature showed no effect of the fan except in the experiment at 27.4°C. when there was a temporary fall of 0.2°C. The skin temperature in this same experiment fell almost 1°. In the range between 28.8°C. and 32.2°C. the skin temperature fell about ½°. At 34.0°C. and above there was the slight drop of 0.3°C. when the fan was started. At 34.7°C. the skin temperature continued to rise in spite of the fan, although a transitory cooling for about 10 minutes was observed. In all experiments the skin temperature showed an immediate fall after the fan was turned on. This was due to the blowing off of the excess moisture on the skin surface. This was followed shortly by a rise in surface temperature to a higher level where the increased air motion played only a relatively small role in the heat loss, affecting neither the convection nor vaporization rates. Air conditioning engineers have long recognized the desirability of intermittent air currents.

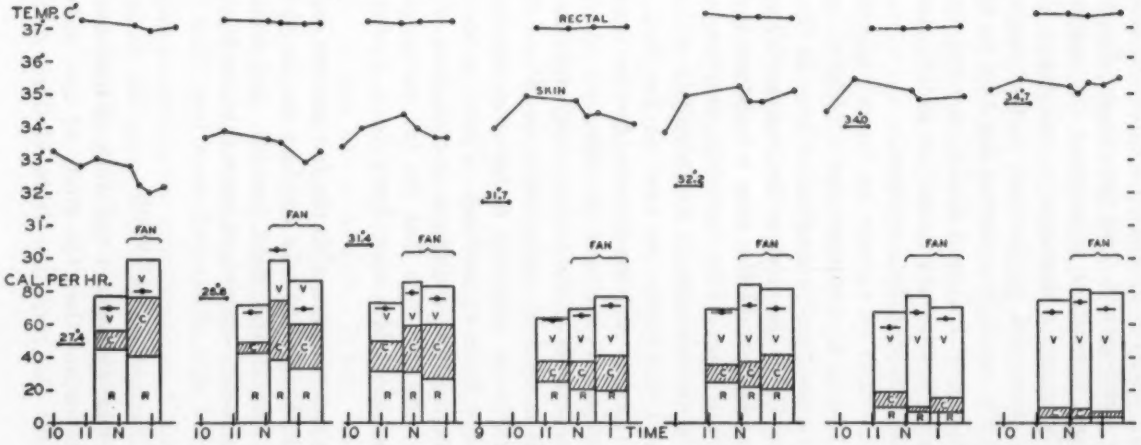

Fig. 1 The effect of forced air currents from an electric fan on metabolism, heat loss, rectal temperature, skin temperature, radiation, vaporization, and convection.

Heat lost by radiation decreased steadily in amount with increasing air temperatures and practically disappeared at 34.7°C. In each individual experiment the cooling of the skin necessarily caused a decrease in radiation. Convection was markedly increased by the fan in the range between 27.4°C. and 30.4°C. until it accounted for 33% to 40% of the total heat lost. At 31.7°C. and 32.2°C. the fan caused only a slight increase in convection, and at 34.0°C. and 34.7°C. convection was even slightly decreased.

Vaporization began to assume an important role in heat loss at 31.7°C., and at temperatures of 34.0°C. and above took care of practically all heat elimination. In the temperature range over 28°C. the electric fan increased vaporization although after blowing off the excess moisture the increase was small, about 10%. The humidity was low enough to insure adequate vaporization in the relatively still air of the calorimeter, and there was no need of the fan. In very humid air at the higher temperatures the fan might be of service in renewing the air near the skin.

The two experiments at the highest temperatures are most instructive. It is evident under these conditions that when the air temperature is within 1° of the skin temperature the fan accomplishes little or nothing. There is no change in vaporization, convection, radiation, or total heat elimination. The reports of the two men that the fan made them feel a little less uncomfortable may have been purely psychic association.

These seven experiments with the fan answer the question of Barr and Du Bois ('18). In the first six the skin became cooler in the second and third periods, and radiation decreased, but increased convection more than made up for this loss and the cool skin eliminated more heat than the warm skin.

CLOTHING

From a practical standpoint the study of men clothed or in bed is more important than the study of men naked. It is easy to measure the radiation from clothing and bedding

and most clothing materials are good 'black body' radiators in the infra-red spectrum from 5 μ to 20 μ. Unfortunately technical difficulties are greatly increased especially in studying large amounts of bedding because thermal equilibrium is established so slowly. The bedding also prevents free circulation of air in the calorimeter so that the calorimeter values of the heat eliminated may be doubtful. For the above reasons two experiments made on a man with bedding were discarded and our attention turned to rather simple types of closely fitting clothing.

The experimental data of three experiments on subject D are given in table 2. The best results were obtained when the subject D was dressed in ski suits. One of these was the common type of blue woolen material of moderate thickness and of such soft texture that snow clings to it. The jacket, closed with a zipper, fitted snugly and the trousers rather roomy at the hips and knees were tight at the ankles. Underneath the ski suit the man wore thin cotton drawers which came down to the ankles and a thin cotton undershirt with long sleeves. His feet were covered with a pair of thin cotton socks and over these thick woolen ski socks weighing 175 gm. The underclothing weighed 405 gm., the ski suit 1860 gm. The effective radiating surface of the ski suit, feet and head was 1.78 sq.m. and the effective radiating area of the naked man was 1.54 sq.m.

One experiment with exercise was performed in a white cotton suit cut just like a ski suit. It was the type used by track athletes to keep warm before they strip for competition and was made of rather thick material smooth on the outside, fluffy on the inside. The suit weighed 660 gm. The socks and underclothing were the same as those used with the blue ski suit.

The pajamas used in the experiment on February 13, 1935, weighed about 200 gm. A thin sleeveless cotton undershirt was worn under this and the feet were covered with woolen socks.

TABLE 2

Experimental data on clothing experiments

SUBJECT, DATE, WEIGHT, REMARKS	END OF PERIOD	CO_2	O_2	RQ	H_2O	URINARY N. PER HOUR	PULSE RATE	TOTAL HEAT PRODUCED	CALORIMETER TEMPERATURE	CALORIMETER HUMIDITY	RECTAL TEMPERATURE	TOTAL HEAT ELIMINATED	SKIN TEMPERATURE		RADIATION	CONVECTION	VAPORIZATION
													Time	Temperature			
		gm.	*gm.*		*gm.*			*Cal.*		%		*Cal.*			%	%	%
EFDB. April 2, 1937, 76.8 kg.	10.40										36.85		10.22	28.9	67	8	25
Cotton ski suit. 1st period	11.40	23.2	20.1	0.84	34.9		60	67.3	22.41	30	36.70	81.8	11.45	28.8			
basal; last 2 periods mild	12.30	33.4	28.8	0.84	33.4		54	97.1	22.62	33	36.84	83.9	11.50	31.3	67	10	23
exercise	1.05	25.9	23.0	0.82	24.7	0.55	53	77.0	22.90	37	37.06	62.6	12.33	29.3	49	28	23
													1.10	29.0			
EFDB. March 12, 1936, 76.5	10.30												10.00	28.2			
kg. Wool ski suit and wool	11.30	22.6	19.7	0.83	38.5		55	65.7	22.30	37	36.70	83.9	11.34	28.3	66	7	27
socks. Both periods basal	12.30	23.2	20.1	0.84	37.3	0.51	51	67.3	22.28	35	36.64	84.0	12.32	28.0	65	9	26
EFDB. February 13, 1935,	11.44										37.20		10.48	29.3			
76.1 kg. Pajamas. Both	12.44	22.7	20.6	0.80	32.1		55	68.1	25.09	26	37.14	76.8			53	23	24
periods basal	1.44	23.5	20.5	0.84	33.2	0.56	52	68.3	25.08	26	37.02	75.8	1.47	30.1	54	20	26

In spite of the difficulties of technic much can be learned by comparing clothed experiments with naked experiments at the same environmental temperature. Subject D dressed in

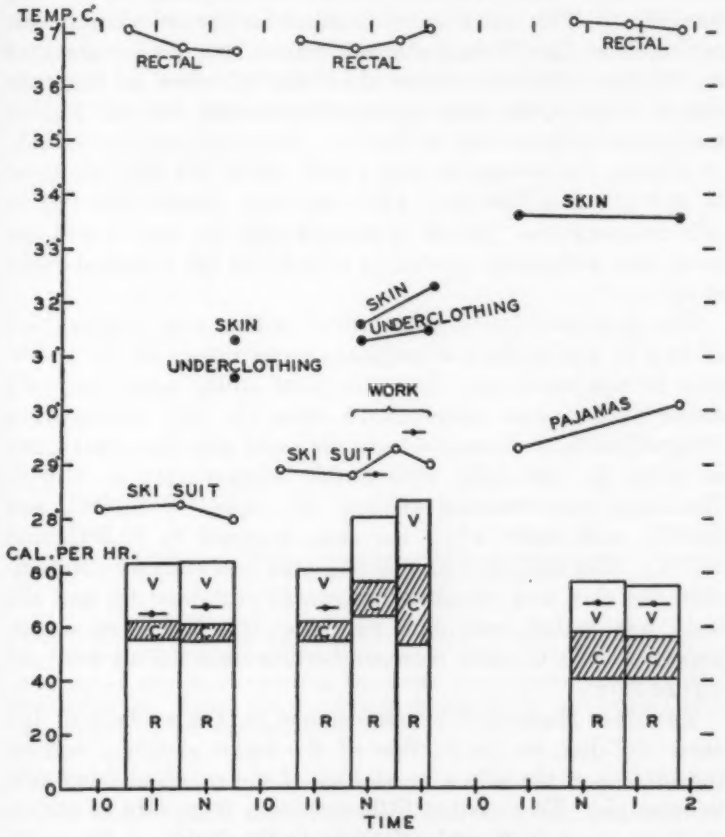

Fig. 2 Heat loss from a clothed subject.

the blue ski suit lay motionless 3 hours in the calorimeter at 22.3. During the first experimental hour he was comfortable but was a little cool the second hour even though the air was 72°F. Just a week before he had been perfectly warm skiing

in exactly this same clothing at a temperature of 16° below zero F. (—29°C.). His skin at the end of the calorimeter experiment had an average temperature of 31.2°C., about a degree above the shivering point; the surface of the suit was 28°C. This same subject naked in the calorimeter experiment at 22.9°C. had started with a skin temperature of 31.7°C. but it fell rapidly to about 29.9°C. when he began to shiver. This same man in experiment with the air 23.4°C. had a chill with the skin at 30.1°C. The other subject, J.D.H., at similar temperatures had a chill when the skin dropped to 30.7°C. The blue and white ski suits slowed the fall in skin temperature. But it is evident that the man would not have been sufficiently protected to prevent the eventual onset of chill.

The pajamas experiment at 25°C. with a skin temperature of 33.5°C. and surface of pajamas temperature of 29° to 30° may be compared with the experiment on the same man (D) naked at the same temperature when his skin temperature dropped to 31.75°C. and he had a chill and with the experiment in which he felt chilly with a skin temperature at 31.8°C. The other experimental subject, H., naked at 25.1°C. and 25.4°C., was chilly when the skin dropped to 32.3°C. and 31.7°C. The subject with the pajamas felt entirely comfortable, the skin and rectal temperatures remained up and the body was losing heat only very slowly. Thus the cotton pajamas at 25°C. offer more protection than the all wool ski suit at 23°C.

The lines showing the temperature on the surface of the outer clothing, on the surface of the under clothing, and on the surface of the skin give an idea of the gradient from skin to outer air. There is but little radiation from skin to underclothing, more from underclothing to the inside of the outer suit, and still more from the surface of the clothing to the walls of the calorimeter. The effective radiating surface of the ski suits is about 16% greater than that of the naked body. In the experiment on D, naked at 22.9°C., the difference between the temperature of the skin and calorimeter walls was

7.0°C. The surface of the ski suit had an average temperature of 28°C. and the thermal difference was 6°C. or 15% less than with the naked man, and this almost exactly balances the greater surface of the clothing. The total radiation thus remains the same under these conditions, with or without clothing. Vaporization at these temperatures is but little changed by ordinary clothing which is porous enough to permit the free passage of water vapor. Convection is not

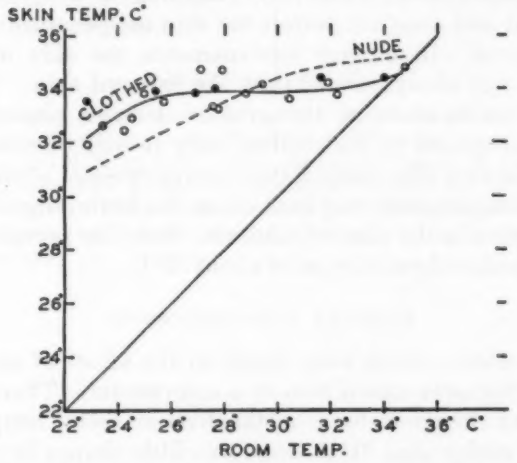

Fig. 3 Effect of clothing upon skin temperature. Circles show average skin temperature under ordinary indoor clothing after the subjects had been sitting quietly for an hour in the prevailing atmosphere. Open circles subject D, solid circles subject H. Dashed line average skin temperature of nude subjects after 2 hours exposure under basal conditions.

greatly changed in quiet experiments with clothing. Motion in the single work experiment caused a great increase in convection just as it does with naked men.

On comparing experiments with the man naked and clothed it appears that when clothing is used the skin corresponds to the subcutaneous tissue of the naked man and loses heat to the outside air through the clothing just as the subcutaneous tissue of the naked man loses heat through the skin. A comparison of the skin temperature under clothing and exposed

is interesting. The subjects always remained at least an hour, before starting an experiment, in ordinary clothing in the prevailing experimental atmosphere. The skin temperature under the clothing was measured as they undressed for the experiment. In the cooler portion of the neutral zone, 28°C. to 30°C., clothing does not affect the skin temperature appreciably, and the skin temperature under the clothing maintains this level (33.8°C.) from 25°C. to 32°C. Thus, in environments lower than 29°C. clothing delays the loss of body heat and does not permit the skin temperature to fall to a chill level. In warmer environments the skin under the clothing was always cooler than the exposed skin. This is a natural consequence of the greater demand placed on the sweat mechanism by the clothed body to help eliminate body heat. The two skin temperature curves cross at about 28.5°C. and this temperature may be taken as the beginning of sensible perspiration in the clothed subjects. Sensible perspiration in the two nude subjects began at about 30°C.

SUMMARY AND CONCLUSIONS

Seven observations were made on the effect of an electric fan blowing over naked men in a calorimeter. There was no significant change in basal metabolism or rectal temperature. With air cooler than 31°C. there was little change in vaporization and radiation but a marked rise in convection which accounted for as much as 33% to 40% of the total heat elimination. In warmer air convection rose less markedly, and in air above 34°C. did not increase at all. At the temperature of 34.7°C. the fan made no change in heat production, heat elimination, radiation, convection, vaporization, rectal temperature, or average surface temperature. It is obvious that with air as warm as the skin (34°C. to 35°C.) and moderate humidity there is no physical benefit to be derived from a fan.

At lower temperatures the fan caused an increase in convection which permitted the body to lose more heat through a cool skin than it had lost in quiet air with a warmer skin.

The effect of clothing was studied in three experiments. Observations made on the clothed man were compared with observations at the same temperature when naked. The surface of the clothes was 1° or 2°C. cooler than the naked skin, but the radiating surface was so much larger that the total radiation was about the same. Vaporization was not changed significantly. The temperature gradient shows a slight drop between underclothing and outer suit, and a still greater drop between the surface of the suit and the walls of the room or the calorimeter.

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SKIN AND BODY TEMPERATURES OF NORMAL INDIVIDUALS UNDER COLD CONDITIONS [1]

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TWO FIGURES

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As a part of an investigation of various physiological processes in schizophrenia, the reaction of the heat-regulating mechanisms to environmental temperature changes has been studied in both schizophrenic and normal subjects. The present investigation deals with the effects on skin and body temperatures of exposure to cold environmental conditions in normal individuals.

The experiment was performed in a psychrometric laboratory in which environmental conditions could be satisfactorily controlled. The skin and body temperatures were measured by thermocouples. The subjects were all studied in the nude under basal conditions, the beds on which they lay being constructed of coarsely woven cane, devoid of any coverings. The skin temperature was measured at nine points, the forehead and eight other areas on the right anterior half of the body surface, namely, the chest, abdomen, upper arm, lower arm, thenar eminence, middle finger, thigh, lower leg, and dorsum of foot. In view of the high degree of similarity of symmetrically located points on the skin (Freeman, Linder and Nickerson, '37), it was felt that an adequate description of the skin temperature could be secured by a study of only one side. Readings were taken

[1] This work was aided by a grant from the Rockefeller Foundation.

30 minutes after recumbency and at three half-hourly periods thereafter, so that the total exposure to the environmental conditions lasted 2 hours.

The effects of two environmental conditions were investigated, viz., 20°C. and 15°C. Ten normal subjects were studied at each temperature level. The figures relating to the levels of temperature and humidity attained during the study are listed in table 1. At the lower temperature, although it was endeavored to make the 5°C. difference the only new variable introduced, significant differences were obtained between the average values and variation of relative humidity and air velocity as well as increased variation in temperature. Such differences may have affected the results to some degree.

TABLE 1

Values pertaining to the environmental conditions attained on the two experimental days (20°C. and 15°C.)

	20°C.		15°C.	
	Mean	Standard deviation	Mean	Standard deviation
Temperature (°C.)	20.13	0.24	15.04	0.44
Relative humidity (%)	21.9	1.56	27.1	2.96
Air velocity (ft./min.)	6.6	4.5	4.3	2.6

The effects of exposure to the experimental conditions upon the levels of skin and rectal temperatures are shown in figure 1. In the case of the skin there is a fall in temperature at almost every location considered. This drop is more marked in the first hour of the experimental day, indicating that some degree of equilibrium is being achieved during the second hour. The case of the forehead is interesting in that the second reading, 1 hour after exposure, is actually higher than that after 30 minutes of exposure, and not until the next reading 90 minutes after exposure is a fall in temperature evident. This initial rise is probably due to the redistribution of blood incident upon changing from the vertical to the horizontal position, the resulting increase in vascularity counteracting the loss of heat to the colder environment and preventing for some time a fall in skin temperature. The same phenomenon has been noted by

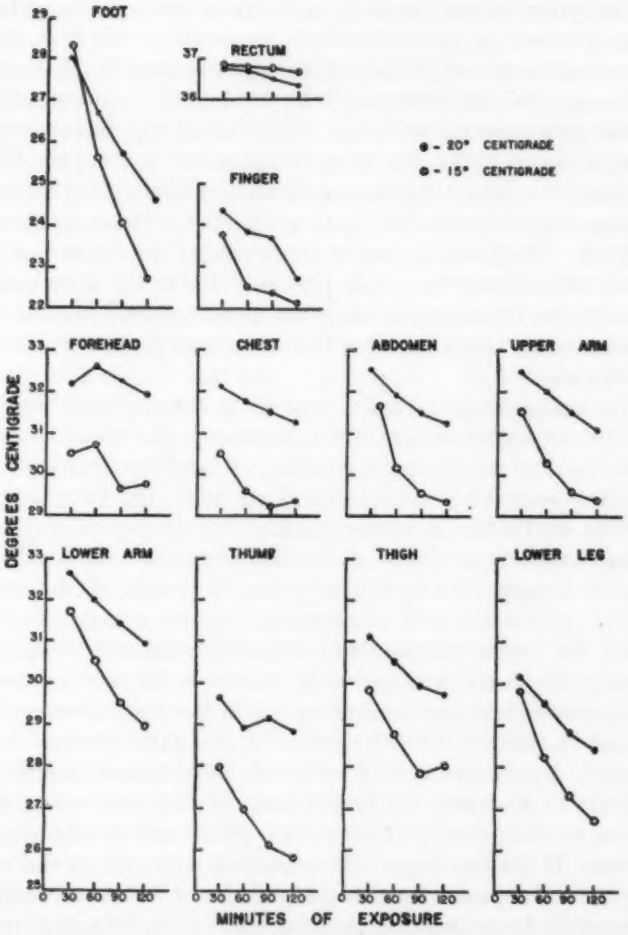

Fig. 1 Graphs illustrating the behavior of the averages of the skin and the rectal temperatures in ten normal subjects at 20°C. and the same number of individuals at 15°C. Four readings were taken at intervals of 30 minutes during a total exposure of 2 hours.

Talbot ('31). The different portions of the body surface cool to a varying degree, the drop in temperature over the 2 hours being greatest in the extremities, particularly the feet, where the change from the initial level is approximately 3.5°C. at an environmental temperature of 20°C. and 5.5°C. at an environmental temperature of 15°C. The initial fall is much more precipitous at 15°C., the drop being of such a degree that a tendency for the establishment of an equilibrium is frequently evident sooner than at 20°C., where the rate of heat loss is more gradual. The lower initial temperature of the fingers as compared with that of the feet is probably due to the fact that previous to the examination they had been exposed to cool ward temperatures while the feet had been kept warm by blankets or slippers.

The rectal temperature shows little change until the third determination, 90 minutes after exposure, the trend becoming more marked at the next reading. The drop in rectal temperature over the 2 hours is 0.4°C. at 20°C., i.e., twice as great as that at 15°C. A comparison of the slopes of the rectal temperatures with those of the skin temperatures is of interest. It is apparent that during the first half of the experimental period the skin temperature shows a marked change while the rectal temperature remains relatively unaffected. During the remaining interval, however, the fall in surface temperature becomes less steep while the rectal temperature begins to show a definite drop. In this difference of trends between the internal and external body temperatures it is difficult to evaluate the importance of the vasomotor mechanism as differentiated from the effects of purely physical forces. If the cooling of the organism were influenced solely by physical factors, the delay in change of the rectal temperature might be considered as a lag due to the interposition between the internal and external portions of the body of layers of varying conductivity. This reasoning, however, does not explain why at 15°C., with an initial rectal temperature essentially the same as that at 20°C., the rectal temperature level falls more slowly than at the higher temperature. It would

seem reasonable that a neurogenic or humoral mechanism must be considered as playing an important role in this reaction.

The vasoconstriction resulting from the cold stimulus decreases the conductivity of the skin and maintains the blood supply in the interior. Heat loss through the skin is thus decreased. Heat production is increased by chemical processes resulting from the discharge of adrenalin. Thus, body temperature is maintained for a time. However, the increased gradient between internal and skin temperatures results in further transfer of heat to the periphery so that heat loss goes on rapidly. As the heat conserving mechanisms fail, the level of body temperature eventually falls and decreases the gradient between it and skin temperature. The diminution in the gradients between the internal and skin temperatures and between the skin and external temperatures results in a slowing of the rate of fall in the skin temperature and the eventual establishment of a state of equilibrium. The lesser fall in body temperature at 15°C. is probably the result both of more intense vasoconstriction and of a greater chemical production of heat. The large surface, lack of adipose tissue, and absence of heat-generating organs, explains the greater fall in temperature of the extremities. In figure 2 are shown the means of the four readings on the ten subjects at each point, measured at both environmental temperatures. From this figure it is evident that the temperatures of the forehead, trunk, and arms hover around the same level; that the temperature of the legs is slightly lower; and that the extremities have the lowest temperature. The legs show a gradient of temperature from the thigh to the foot, while on the upper extremities, the upper and lower arms have the same temperature. The lack of gradation on the arms may be due to their greater proximity to the heart and also possibly to the absorption of heat from the trunk.

In agreement with expectation the average skin temperatures are significantly higher at 20°C. than at 15°C. except for the finger point.

The response of the rectal temperatures, however, to the change in environmental temperature is different from that of the skin temperature. The average level at 15°C. is actually somewhat higher than that at 20°C. although not significantly so.

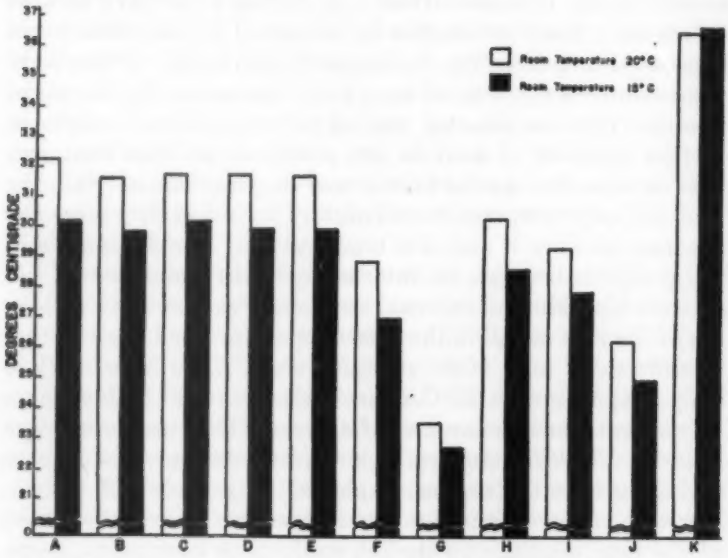

Fig. 2 Averages pertaining to skin and body temperature measurements of four determinations taken at half-hourly intervals over a 2-hour period on each of ten normal subjects at an environmental temperature of 20°C. and the same number of different subjects at 15°C. The letters refer to the points at which the temperature was measured, viz., A, forehead; B, chest; C, abdomen; D, upper arm; E, lower arm; F, thenar eminence; G, middle finger; H, upper leg; I, lower leg; J, foot; K, rectum.

In view of the fact that the averages shown in figure 2 give no information concerning the variation of individuals about them nor as to the change from period to period, analysis of variance has been carried out to determine these.

In table 2 are given standard deviations pertaining to the variation between periods, among individuals, and within individuals. The between period standard deviations illustrate

the variation of the four half-hourly period means around the averages of the group shown in figure 2. Each value is thus a measure of the change in skin or body temperature resulting from exposure to the experimental conditions. As is evident from figure 1, the reaction of the organism is a fall in temperature. Consequently the more pronounced the cooling effect the greater is the variation about a given level of temperature and the higher is the magnitude of the values. The

TABLE 2

Standard deviations of skin and body temperature measurements pertaining to differences between periods, between individuals, and within individuals. Four determinations on each of ten normal subjects at an environmental temperature of 20°C. and the same number on ten different subjects at 15°C.

SKIN POINT	A [1]		B [1]		C	
	BETWEEN PERIODS		BETWEEN INDIVIDUALS		WITHIN INDIVIDUALS	
	20°C.	15°C.	20°C.	15°C.	20°C.	15°C.
Forehead	0.90	1.66	1.32	2.14	0.50	0.61
Chest	1.14	1.83	0.92	2.11	0.30	0.63
Abdomen	1.82	3.25	1.02	1.89	0.25	0.66
Upper arm	1.92	3.00	0.90	1.46	0.32	0.48
Lower arm	2.22	3.82	1.20	1.31	0.35	0.47
Thumb	2.47	4.50	3.03	2.41	0.90	1.20
Finger	2.17	2.30	3.06	3.13	1.20	1.25
Thigh	1.97	2.92	1.09	2.36	0.35	0.79
Lower leg	2.51	4.25	1.62	1.66	0.28	0.39
Foot	4.53	7.56	3.73	2.02	0.53	0.80
Rectal	0.625	0.272	0.298	0.783	0.138	0.127

[1] Figures in these columns denote significance.

figures in table 2 A are practically all significant when compared with the net intra-individual variation (table 2 C), showing that the cooling effect has been greater than chance variation. As we have seen in figure 1, the forehead is the most constant in temperature while the feet cool to the greatest degree. The more marked fall in temperature at 15°C. is shown by the increased magnitude of the values. The greater stability of the rectal temperature at 15°C. is reflected quite clearly by its lower value.

In table 2 B are shown the standard deviations pertaining to the variation of the individual means about the averages of the group. All these values pertaining to the inter-individual variation are significant when compared with the net intra-individual variation as given in table 2 C. This implies that individuals on the whole have inherently different temperature levels. The values are somewhat larger at 15°C. than at 20°C., implying that decreased temperature increases the difference between individuals.

In general the greatest differences between individuals are found on their extremities. It is of interest that the rectal temperature at 15°C., despite its lesser fall in temperature, shows much greater variation among individuals than it does at 20°C., thus reflecting the trends of the skin temperatures.

The measures of variation obtaining between individuals and between periods yield no information as to the behavior of the individual. Furthermore we need a quantity which may serve as a basis of reference for determining the significance of the preceding measures of variation. Such a quantity is the net intra-individual standard deviation of table 2 C to which reference has already been made. This quantity measures the variation existing, on the average, within the individual after the variation due to cooling has been removed. Thus this net intra-individual standard deviation also measures the extent to which individuals differ from each other in the cooling curves. The greater are the values the greater are the individual differences. The greatest individual differences in cooling curves are found on the hands and, to a lesser degree, on the feet. The values at 15°C. are generally higher than those at 20°C., implying that at the lower temperature there is an increased heterogeneity in behavior.

The data were further analyzed to determine whether any of the following relationships existed: a) between the initial skin temperature and the rate of cooling of the skin; b) between the initial rectal temperature and the rate of cooling of the skin; c) between the average levels of skin and rectal temperatures for the various individuals; d) between the

levels of skin and rectal temperature for a given individual on the average.

No well-marked relationship was observed between the initial temperature of the skin and its rate of cooling. For approximately two-thirds of the locations those individuals having the higher initial skin temperature cooled somewhat more markedly but the relationship appears to be so weak that little emphasis can be laid upon it.

The subjects were divided into two groups having high and low initial rectal temperatures. The cooling curves for the various skin points were also separated on this basis. An analysis of variance performed on the data by this dichotomy did not show any significant relationship. Presumably the initial body temperature cannot serve as an indication of the subsequent course of the skin temperature under the experimental conditions utilized here.

The relationship between the average levels of skin and rectal temperature for the various individuals is very slight and, surprisingly, in a negative direction. Among individuals it averaged — 0.13 for both environmental temperatures, and within individuals, — 0.24. The finding of high rectal temperatures with low skin temperatures in normal individuals would imply an active means of maintaining a gradient of heat between the interior and the periphery of the body. While this relationship is ill-defined, it has been shown by other investigators.

Heiser and Cohen ('33) reported a negative relationship between the temperatures of the left wrist and that of the mouth. Steele ('34) noted that the diurnal variation in the temperature of the extremities was opposite to that of the rectal temperature. Burton ('35) found little correlation between the rectal and surface temperatures but in fifteen cases out of forty the changes in these components occurred in opposite directions.

SUMMARY

A study of the skin and rectal temperatures of ten normal subjects exposed to an environmental temperature of 20°C. for 2 hours and an equal number of subjects at a temperature of 15°C. shows the following results:

1. At both environmental temperatures the skin temperatures fall markedly, more rapidly in the first hour and more precipitously at 15°C. than at 20°C. The fall is least on the forehead and greatest on the extremities.

2. The rectal temperature shows little change for an hour but after that begins to fall, more rapidly, however, at 20°C. than at 15°C., the mean level being higher at the latter temperature.

3. Individuals vary from each other significantly as to their temperature levels and differ from each other to the greatest extent on the extremities.

4. The rate of fall in skin temperature is independent of the initial level of the rectal temperature and is only slightly influenced by the initial skin temperature.

5. The average levels of skin and rectal temperatures are slightly related to each other but in a negative direction.

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THE INFLUENCE OF THE DIET AND ENERGY INTAKE UPON ACUTE VITAMIN B_1 DEFICIENCY IN THE RAT[1]

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Although there has been much work reported on the effect of a vitamin B complex deficiency and of a simple vitamin B_1 deficiency in the rat, only scattered reports can be found concerning the actual production of the neuromuscular symptoms associated with acute vitamin B_1 deficiency. Hence it appears that the rat has been found to be more resistant to vitamin B_1 depletion than has the pigeon, the chick, and the dog, as Cowgill ('34) has suggested. In order to determine whether this apparent resistance has been due to the dietary procedure used, a study has been made of the influence of the diet and the method of feeding upon the rat during vitamin B_1 deficiency. Because both the nature of the non-protein constituents of the diet and the method of feeding influenced the results, a study was also made of the energy intake. It is the purpose of this paper to report these results.

EXPERIMENTAL PROCEDURE

The rats came from a Wisconsin strain colony which received the standard stock diet used in this laboratory (Prickett, '34). When started on experiment, they were from 23 to 26 days of age and averaged about 55 gm. in weight. Individual metal cages equipped with false bottoms of hardware

[1] Published with the permission of the Director of the Alabama Experiment Station.

cloth, having 3 meshes to the inch, were used in all experiments. The animals were weighed weekly and at development of the symptoms of acute vitamin B_1 deficiency or at death. The criterion selected as evidence of acute vitamin B_1 deficiency was the development of neuromuscular symptoms, which have been described in detail by Church ('35) and to a lesser extent by Prickett ('34).

All basal diets contained constant amounts of protein, salt mixture, and agar per Calorie, while the non-protein source of energy was varied (table 1). Three different carbohydrates and one fat were used; the carbohydrates were sucrose, cornstarch, and dextrinized cornstarch, and the fat was coconut fat. In three diets (3 G, 3 B, and 3 D) the carbohydrate furnished all of the energy except that furnished by the protein and the daily supplements. In three other diets (23 C, 23 B, and 23 D) about one-half of the energy from carbohydrate was replaced by fat, and in another single diet about three-fourths of the energy from sucrose was replaced by fat (diet 40 C). In a fourth instance, casein purified by re-precipitation (diet 3 G-1) was compared with the extracted casein (diet 3 G). These caseins were compared in only one diet inasmuch as no great difference was found in the results. The diets were prepared at frequent intervals, and the fat-containing diets were stored at 3°C., except for the short period each day when the rats were being fed.

Two methods of feeding were employed. In one, the rats were fed ad libitum after the first 3 days (ad libitum group). An attempt was made to feed these rats only such an amount of diet as would be consumed in the succeeding 24 hours. In the other method, the animals were given the same amount of energy (limited group); this was an amount which satisfied the maintenance requirements and allowed a slow rate of growth; after the third day, these rats received 12 + Cal. per day. Any excess diet was weighed back weekly, and upon development of neuromuscular symptoms or death in both groups. In most cases, all rats received the same energy allowance for the first 3 days; namely, 6.25, 9.16, and 9.16 Cal.

for the first, second, and third days, respectively. Such a procedure allowed the animals to become adapted to the diets and thus minimized intestinal disturbances. The energy values of the diets were calculated on the basis of 4.0 Cal. per gram for the carbohydrates, autoclaved yeast, and protein, and of 9.0 Cal. per gram for the coconut fat, cod liver oil, and linseed oil. Besides the rats placed on vitamin B_1 deficient

TABLE 1

Percentage composition of basal diets[1]

INGREDIENTS	3 G	3 G-1	3 B	3 D	23 C	23 B	23 D	40 C
Casein (extracted)[2]	18.0	...	18.0	18.0	23.0	23.0	23.0	27.0
Casein (reprecipitated)[3]	...	18.0	...	...	...	...	...	...
Coconut fat[4]	...	...	...	...	23.0	23.0	23.0	40.0
Sucrose[5]	77.0	77.0	...	...	47.5	...	...	25.5
Cornstarch[6]	...	...	77.0	...	...	47.5	...	...
Dextrinized cornstarch[7]	...	...	...	77.0	...	...	47.5	...
Agar	1.0	1.0	1.0	1.0	1.3	1.3	1.3	1.5
Salts 186[8]	4.0	4.0	4.0	4.0	5.2	5.2	5.2	6.0

[1] Each rat received 0.25 gm. autoclaved yeast (J. Nutrition, vol. 13, p. 477, '37), 0.1 cc. cod liver oil, and 0.1 cc. raw linseed oil daily as separate supplements.

[2] J. Nutrition, vol. 8, p. 1, '34.

[3] Prepared by twice dissolving in NH_4OH and precipitating with acetic acid. After each precipitation, the casein was washed by percolation with water until the washings were neutral. After the final washing, it was partially dehydrated by washing with 95% alcohol, dried at 40 to 45°C., and ground.

[4] Pu-Re-Co brand, Capital City Products Co., Columbus, Ohio.

[5] Commercial granulated sugar.

[6] Argo brand, Corn Products Refining Co., New York.

[7] Cornstarch, thoroughly moistened and autoclaved for 5 hours at 15 pounds pressure, dried at 60 to 70°C., and ground.

[8] J. Biol. Chem., vol. 89, p. 199, '30.

diets, control rats were fed certain of the basal diets and adequate vitamin B_1.

All rats received daily supplements of 0.1 cc. of cod liver oil, 0.1 cc. of raw linseed oil, and 0.25 gm. of autoclaved yeast. These supplements were given in a separate container, except in the limited feeding where the autoclaved yeast was mixed into the basal diets at such a level that each rat would receive 0.25 gm. in the daily food. The control rats received adequate

vitamin B_1 as 20 mg. of a fuller's earth adsorbate of a brewer's yeast extract on alternate days (Salmon and Goodman, '37). Distilled water was always available.

RESULTS

Effectiveness in producing neuromuscular symptoms

The nature of the non-protein constituents had much more influence on the effectiveness of the diets in producing the neuromuscular symptoms of acute vitamin B_1 deficiency than did the method of feeding (table 2). The combined data show that the dextrin and starch diets (3 D and 3 B) were least effective, the sucrose, the dextrin-fat, and the sucrose-high fat diets (3 G, 23 D, and 40 C) were next, and the starch-fat and sucrose-fat diets (23 B and 23 C) were most effective. The percentages of rats developing neuromuscular symptoms were 44, 46, 65, 67, 69, 90, and 93 for diets 3 D, 3 B, 3 G, 23 D, 40 C, 23 B, and 23 C, respectively.

Although fewer rats were subjected to the ad libitum than to the limited method of feeding, the ad libitum procedure appeared to be somewhat more effective in producing the symptoms than did the limited procedure (table 2). This was especially true for diets 3 D, 23 D, and 40 C and may have been caused by the longer time these rats were on experiment. Certainly, other deficiencies, as well as the low level of food intake, would affect the rats adversely in the longer experimental periods.

The sex of the rats did not seem to have any especial influence on the development of acute vitamin B_1 deficiency. A somewhat higher percentage of females (74%) than of males (69%) exhibited neuromuscular symptoms, but it is considered that this difference was not significant. These percentages are based on a total of 223 female and 274 male rats. Patras and Templeton ('33) have reported similar results.

TABLE 2

The development of neuromuscular symptoms and the weight changes of rats in acute vitamin B_1 deficiency as influenced by the diet and method of feeding

SERIES	SUPPLEMENT	DIET	NUMBER OF RATS		INITIAL WEIGHT		FINAL WEIGHT		MAXIMUM WEIGHT		TIME MAXIMUM WEIGHT ATTAINED		TIME TO	
			Total	Developing N.M.S.[1]	Developing N.M.S.[1]	Death no N.M.S.[1]	Developing N.M.S.[1]	Death no N.M.S.[1]	Developing N.M.S.[1]	Death no N.M.S.[1]	Developing N.M.S.[1]	Death no N.M.S.[1]	N.M.S.[1]	Death no N.M.S.[1]
					gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*days*	*days*	*days*	*days*
Limited (isocaloric)[2]	None	3 G	122	78	58	57	45	40	59	57	14	2	52	43
		3 G-1	18	16	55	53	43	41	59	55	17	14	51	41
		3 B	45	20	57	55	43	38	59	56	12	3	50	43
		3 D	28	7	52	55	49	44	72	62	76	46	119	123
		23 C	101	95	56	58	51	39	60	58	22	0	68	51
		23 B	29	27	57	54	51	46	62	61	25	45	59	79
		23 D	28	17	58	59	50	41	66	62	59	36	129	77
		40 C	15	8	53	55	48	45	58	61	47	61	105	138
	Vitamin B_1[3]	3 G	39	..	..	56	..	51[4]	..	..	..	..	..	..
		3 B	11	..	..	53	..	44[4]	..	..	..	..	..	..
		23 C	28	..	..	56	..	57[4]	..	..	..	..	..	..
Ad libitum	None	3 G	17	12	54	58	44	42	61	62	10	8	41	31
		3 G-1	12	10	55	59	44	40	62	66	11	10	44	41
		3 B	12	6	56	55	49	40	67	61	15	8	44	35
		3 D	17	13	57	54	63	38	82	60	40	10	71	32
		23 C	21	19	55	57	52	46	68	69	19	14	42	46
		23 B	12	10	55	50	58	41	74	66	25	14	49	29
		23 D	12	10	55	54	71	55	84	58	43	3	62	11
		40 C	11	10	57	58	75	92	87	95	69	147	85	171
	Vitamin B_1[3]	3 G	13	..	..	57	..	92[4]	..	..	..	..	..	..
		3 B	5	..	..	52	..	94[4]	..	..	..	..	..	..
		23 C	4	..	..	53	..	108[4]	..	..	..	..	..	..

[1] The neuromuscular symptoms of acute vitamin B_1 deficiency.

[2] Each rat received 12+ Cal. per day after the third day.

[3] J. Nutrition, vol. 13, p. 477 ('37). Each rat received 20 mg. on alternate days of a fuller's earth adsorbate of a brewer's yeast extract.

[4] Weight at 42 days.

Time to development of neuromuscular symptoms

On every diet, subjecting the rats to the limited method of feeding increased the time to the onset of neuromuscular symptoms (table 2). This increase in the time was greater for the fat-containing diets (23 C and 23 B) than it was for the high carbohydrate diets (3 G and 3 B), suggesting that this level of coconut fat exerted a 'sparing' effect on vitamin B_1 when the rats were fed by the limited method. The 'sparing' effect of the coconut fat was found in the high fat diet (40 C) in the ad libitum as well as in the limited method of feeding.

A decided increase occurred in the time required for rats to develop neuromuscular symptoms when dextrinized cornstarch (diets 3 D and 23 D) was substituted for the original cornstarch in diets 3 B and 23 B (table 2). Again, the greatest increases in time occurred in the limited feeding procedure. The dextrin appeared to exert about as great a 'sparing' effect on vitamin B_1 as did 40% of coconut fat.

There appeared to be a relation between the effectiveness of a diet in producing the neuromuscular symptoms and the time of appearance of the symptoms; the diets and method of feeding that produced the neuromuscular symptoms in the shortest and longest times were least effective in producing them in a large percentage of individuals, while the diets and method of feeding that produced the symptoms in intermediate times were most effective. Even then there were exceptions, but these occurred mainly in the fat-containing diets and in the ad libitum method of feeding. Thus, although the fat did not exert a 'sparing' effect in the lower level on the ad libitum feeding, it did materially influence the effectiveness of a diet in producing the neuromuscular symptoms of acute vitamin B_1 deficiency.

The inclusion of fat or of dextrin in the diet or the use of the limited feeding method widened the spread of distribution of the rats which developed neuromuscular symptoms; the greatest concentration in the incidence of distribution was nearest to the average incidence of neuromuscular symptoms

on the ad libitum feeding procedure. Even in those diets which were most effective in producing neuromuscular symptoms of acute vitamin B_1 deficiency, there was a considerable spread.

Some work was also done on the relation of autoclaved yeast dosage to the development of neuromuscular symptoms because a previous report from this laboratory (Salmon and Goodman, '37) indicated that, although the yeast was autoclaved moist for 8 hours at 120°C., it still contained traces of vitamin B_1. Three litters of six rats each were fed diet 3 G-1 (table 1); twelve rats received the usual 0.25 gm. of autoclaved yeast daily and six litter mates received 0.5 gm. daily. Ten of the rats on the lower level of yeast developed neuromuscular symptoms in an average of 56 days and four of the rats on the higher level developed symptoms in an average of 68 days. The increase in time to onset of symptoms on the higher level could be considered as evidence supporting the conclusion reached by Salmon and Goodman ('37). This increase in the time by doubling the autoclaved yeast allowance was of about the same degree as was secured by replacing about 50% of the energy from sucrose by that from coconut fat (diet 23 C) on the same (limited) feeding procedure.

Weight changes

Very little difference was found in the maximum weights of the rats that developed neuromuscular symptoms on all diets in the limited feeding procedure, with the exception of the rats which were fed the dextrin diets, 3 D and 23 D (table 2). These rats gained more weight than the rats on the other diets, despite the fact that all rats received the same energy allowance.

Similar but less uniform results were secured from the ad libitum feeding. In most cases, these rats reached a greater maximum weight than the limited fed rats. The rats which received diet 40 C had the greatest maximum weight, but those that were fed diets 3 D and 23 D were not much lighter.

The weight loss (difference between maximum and final weights) of the rats developing neuromuscular symptoms was somewhat greater and more rapid in the ad libitum than in the limited feeding (table 2). The greatest losses in weight occurred on the high carbohydrate diets (3 G, 3 B, and 3 D), and the least losses occurred on the high fat diet (40 C). With the exception of diet 40 C, the diets and method of feeding which induced the most rapid losses in weight were the least effective in producing the neuromuscular symptoms of the acute deficiency.

Growth was essentially the same in the limited feeding method for the rats that received vitamin B_1 as it was for those not receiving the vitamin (table 2). This made it appear that the rats not receiving the vitamin were utilizing the food about as efficiently as were those that received the vitamin supplement. However, it was always observed in the paired, as well as limited and isocaloric, feeding that the animals which received vitamin B_1 could not be reduced to the same level of food intake as could the deficient rats without causing death.

The weight records of the ad libitum fed rats which received vitamin B_1 show that these rats did not grow as rapidly as rats on stock diet (table 2). Even 0.5 gm. of untreated yeast daily per rat did not give normal growth.

Energy intake relations

The energy intake required for the development of the neuromuscular symptoms of acute vitamin B_1 deficiency was markedly influenced by the non-protein constituents of the diet (table 3). Although the energy intake was similar for the high sucrose and high starch diets (3 G and 3 B), partially substituting coconut fat or dextrin for the sucrose or cornstarch materially increased the energy requirement for the production of neuromuscular symptoms. This was especially pronounced when three-fourths of the non-protein energy in the high sucrose diet (3 G) was replaced by coconut fat (diet 40 C), or when the cornstarch in diets 3 B and 23 B was replaced by dextrin (diets 3 D and 23 D).

TABLE 3

The energy intake of rats in vitamin B_1 deficiency as influenced by the diet and method of feeding

SERIES	DIET	NUMBER OF RATS	TIME TO		TOTAL ENERGY		DAILY ENERGY	
			N.M.S.[1]	Death no N.M.S.[1]	Allotted	Consumed	Allotted	Consumed
			days	*days*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
Limited (isocaloric)[2]	3 G	78	51.6	...	613	535	11.87	10.36
	3 G-1	16	50.6	...	601	522	11.87	10.31
	3 B	20	50.4	...	598	544	11.87	10.79
	3 D	7	118.7	...	1420	1190	11.97	10.02
	23 C	95	67.6	...	805	788	11.91	11.66
	23 B	27	59.3	...	705	681	11.89	11.49
	23 D	17	127.1	...	1522	1388	11.97	10.92
	40 C	8	104.7	...	1252	1227	11.96	11.72
Ad libitum	3 G	12	40.6	...	...	465	....	11.45
	3 G-1	10	43.6	...	...	485	....	11.11
	3 B	6	43.7	...	...	583	....	13.34
	3 D	13	71.4	...	...	1415	....	19.82
	23 C	19	42.1	...	...	679	....	16.14
	23 B	10	49.1	...	...	848	....	17.26
	23 D	10	62.0	...	...	1414	....	22.81
	40 C	10	84.7	...	...	1515	....	17.77
Limited (isocaloric)[2]	3 G	44	...	43.5	515	425	11.84	9.77
	3 G-1	2	...	40.7	481	380	11.83	9.34
	3 B	25	...	43.4	514	433	11.84	9.97
	3 D	21	...	122.6	1467	1360	11.97	11.09
	23 C	6	...	51.5	611	598	11.87	11.60
	23 B	2	...	79.5	948	928	11.93	11.67
	23 D	11	...	77.0	918	895	11.93	11.62
	40 C	7	...	138.3	1656	1605	11.98	11.61
Ad libitum	3 G	5	...	31.0	...	437	....	14.10
	3 G-1	2	...	41.0	...	423	....	10.33
	3 B	6	...	35.3	...	422	....	11.95
	3 D	4	...	32.2	...	551	....	17.12
	23 C	2	...	46.2	...	684	....	14.80
	23 B	2	...	29.5	...	418	....	14.17
	23 D	2	...	11.5	...	286	....	24.87
	40 C	1	...	171.0	...	3408	....	19.93

[1] The neuromuscular symptoms of acute vitamin B_1 deficiency.

[2] Each rat received 12+ Cal. per day after the third day.

The method of feeding influenced the energy intake of the rats that developed neuromuscular symptoms to a lesser extent than the diet (table 3). The greatest difference in caloric intake on the two feeding methods was found on those diets which required the longest time for development of neuromuscular symptoms.

With the exception of diet 40 C, the two diets which were most effective in producing the neuromuscular symptoms of acute vitamin B_1 deficiency in the limited feeding procedure (diets 23 C and 23 B) gave the nearest to the allotted daily energy intake. The diet which was eaten in the least amount (diet 3 D) was the least effective.

Food was consumed more uniformly by the limited (isocalorically) fed than by the ad libitum fed rats, especially in the latter stages of the deficiency; that is, the ad libitum fed rats reduced the food intake much more drastically shortly before the development of neuromuscular symptoms than the limited fed rats. Whereas the limited fed rats were forcibly subjected to a continuous or chronic inanition due to the low level of food intake, the ad libitum fed rats voluntarily subjected themselves to a more acute inanition in the latter stages of the deficiency.

In general, the rats that died without developing the neuromuscular symptoms consumed less total and daily energy than did the rats on the same diet that developed the symptoms of the acute deficiency (table 3). Again the data do not bring out a point that may be significant, namely, that the rats which died without developing neuromuscular symptoms usually reduced their food intake earlier than the rats which did develop neuromuscular symptoms. This was especially true of the rats on diets 3 G and 3 B.

DISCUSSION

These experiments demonstrate that it is possible to produce consistently the neuromuscular symptoms of acute vitamin B_1 deficiency in young rats. On certain diets, at least 90% of the rats developed neuromuscular symptoms. The

most effective diet studied was one in which about one-half of the energy from non-protein sources was furnished by coconut fat and one-half by either sucrose or cornstarch. As a whole, the least effective diets were those in which all of the energy from non-protein constituents was supplied by carbohydrate. This was especially true in the limited method of feeding. These results are essentially in agreement with those of Braddon and Cooper ('14 a, '14 b), and Ariyama ('34).

The time required for the development of neuromuscular symptoms of acute vitamin B_1 deficiency in young rats was influenced by the method of feeding as well as by the nature of the non-protein constituents of the diet. As compared with the results obtained from the ad libitum method of feeding, subjecting the rats to a limited energy allowance increased the time on all diets. Moreover, the diets containing sucrose or cornstarch with one-half of the energy from non-protein sources supplied by coconut fat gave an increase in the time to acute vitamin B_1 deficiency over that obtained from the carbohydrates alone in the limited feeding method, whereas no such effect occurred in the ad libitum feeding method. This result would suggest a 'sparing' action of the fat on vitamin B_1 on the low level of fat when the rats were fed at a low level of energy intake. Replacement of three-fourths of the energy from non-protein sources by coconut fat gave a fat 'sparing' action even in ad libitum feeding. However, the same result was obtained in the diets in which cornstarch was replaced by dextrinized cornstarch; in the ad libitum feeding, the time to acute vitamin B_1 deficiency was increased to about the same extent for the high dextrin diet (3 D) as for the high fat diet (40 C). These results with dextrin seem to be in agreement with those reported by Guerrant et al. (Guerrant and Dutcher, '34 a, b; Guerrant, Dutcher and Tomey, '35; and Guerrant, Dutcher and Brown, '37) on the vitamin B complex.

Postulation of a relationship between the food or energy intake and vitamin B_1 requirement was advanced long before it was known that vitamin B_1 was only one constituent of a complex (Funk, '14; Braddon and Cooper, '14 a, b; Green, '18;

and Plimmer, '26). Recently, added proof that this relationship was actually concerned with the B_1 fraction of the vitamin B complex has been advanced by Amantea ('33, '34, and '35) and by Cowgill ('34). These latter workers have developed formulas, based on the animal weight and food intake, for the determination of the onset of acute vitamin B_1 deficiency or of the vitamin B_1 requirement for growth. The results of the experiments reported in this paper do not deny that there is a direct relationship between the food intake and the production of acute vitamin B_1 deficiency in the young rat, but emphasize that the nature of the diet and the plane of nutrition influence this relationship. Only the non-protein constituents of the diets were varied in these experiments; increasing the fat (coconut fat) up to 40% or substituting dextrinized cornstarch for the original cornstarch more than doubled the energy requirement for the development of neuromuscular symptoms. The plane of nutrition had a less pronounced effect: the energy required for the production of the acute symptoms was similar on certain diets whether the rats were fed ad libitum or at a limited level, whereas on other diets one method of feeding required more energy intake than did the other. These results show, therefore, that any formula devised for calculation of the vitamin B_1 requirement for energy utilization must take into account the source of the energy as well as the plane of nutrition.

SUMMARY AND CONCLUSIONS

1. The effectiveness of a diet in producing the neuromuscular symptoms of acute vitamin B_1 deficiency in the young rat varied decidedly with the nature of the non-protein constituents of the diet, all other components of the diet remaining constant. The most effective diets studied were those in which carbohydrate (sucrose or cornstarch) and fat (coconut fat) each furnished approximately half of the non-protein source of energy. About 90% of the rats on these diets developed neuromuscular symptoms. The method of feeding had less influence than the diet, although the ad libitum method was

somewhat more effective than a limited (isocaloric) method in which each rat received 12+ Cal. per day.

2. The time to onset of neuromuscular symptoms was influenced by both the method of feeding and the nature of the non-protein constituents of the diet. The time was greater for the limited than for the ad libitum feeding method. Replacement of one-half of the energy from carbohydrate (sucrose or cornstarch) by that of coconut fat had no effect in the ad libitum feeding but increased the time in the limited feeding procedure. Replacement of cornstarch by dextrinized cornstarch or of three-fourths of the sucrose in the sucrose diet by coconut fat (energy basis) increased the time in both feeding methods; the effect was as great for dextrin as for the coconut fat.

3. The rats on the limited feeding procedure usually attained a lesser maximum weight and the loss from the maximum to the final weight was less rapid and of a lesser degree than for the rats on the ad libitum feeding procedure. Likewise, anorexia was less acute with the limited method of feeding, especially for those diets which were most effective in producing the neuromuscular symptoms.

4. The nature of the non-protein constituents of the diet and, to a lesser degree, the method of feeding, influenced the total energy required for the production of neuromuscular symptoms. The substitution of dextrinized cornstarch for cornstarch in the high starch diet or of coconut fat for about three-fourths of the energy from sucrose in the high sucrose diet more than doubled the energy intake before the onset of neuromuscular symptoms.

5. If the proper diet is used, the incidence of the neuromuscular symptoms of acute vitamin B_1 deficiency is so consistent in young rats that it is suggested that these symptoms constitute the basic criterion of acute vitamin B_1 deficiency in the rat, as well as in the pigeon and the chick.

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THE QUANTITATIVE REQUIREMENT OF THE GROWING CHICK FOR MANGANESE[1]

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Deficiency of manganese in the feed of chicks (Wilgus, Norris and Heuser, '36, '37) results in a leg disorder known as slipped tendon or perosis. This condition is characterized by a bowing of the leg at the tibia-metatarsal joint, enlargement with a tendency toward flattening of the joint and finally slipping of the Achilles tendon from its normal position. Recent investigations (Gallup and Norris, '37 a, '37 b); Heller and Penquite, '37; Schaible, Bandemer and Davidson, '37; Lyons, Insko and Martin, '37; and Insko, Lyons and Martin, '37) have confirmed the earlier work on the necessity of manganese for the prevention of this abnormality. The experiments reported in this paper were designed to determine the level of manganese most satisfactory for growth and for the prevention of perosis.

All lots of chicks were wing banded at random as described by Wilcke ('36), weighed individually and placed in wire-screen-floored, hot-water-heated battery brooders. The all-mash ration and distilled water were given ad libitum, the water in glazed earthenware fountains. The basal all-mash ration was composed of ground yellow corn 62.5, dried skim-milk 20.0, meat scrap 6.0, liver meal 5.0, soybean oil 3.0,

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sardine oil 1.0, steamed bone meal 2.0, and salt 0.5 parts by weight. This basal all-mash contained 1.56% calcium, 1.03% phosphorus, and 7 ppm. (parts per million) of manganese in the first experiment and 1.67% calcium, 1.01% phosphorus and 6 ppm. of manganese in the second.

All chicks were weighed and observed individually at weekly intervals. The degree of bowing of the legs and the position of the Achilles tendon were determined. The degrees of bowing were recorded as 1, 2, 3 and 3.5 where 1 represented a straight leg (normal) and 3.5° of bowing hardly distinguishable from slipped tendon. The point of the bow is the tibia metatarsal joint, not the diaphysis of the metatarsus.

FIRST EXPERIMENT

Eight lots of day-old Rhode Island Red chicks containing fifty chicks each were started in this experiment. At the end of 1 week thirty-five chicks from each lot were selected for inclusion in the complete experiment. The basal all-mash containing 7 ppm. was supplemented by adding manganese sulfate ($MnSO_4 \cdot 2H_2O$), as follows: lot 1, none; lot 2, 10 ppm. of manganese; lot 3, 20 ppm. of manganese; lot 4, 30 ppm. of manganese; lot 5, 40 ppm. of manganese; lot 6, 60 ppm. of manganese; lot 7, 80 ppm. of manganese; and lot 8, 100 ppm. of manganese. The results of this experiment are shown in table 1.

The quantity of manganese in the ration influenced the percentage of slipped tendons inversely. Seventy-seven per cent of the chicks of lot 1 receiving the basal ration, containing 7 ppm. of manganese, had slipped tendons. Fifty-four per cent of the chicks receiving 17 ppm. of manganese had slipped tendon, while of those receiving 27 ppm. of manganese, about 25% had slipped tendon. Manganese at higher levels entirely prevented slipped tendons, with the exception of one chick in lot 6. This will be discussed later.

The average degree of bowing is also influenced inversely by the amount of manganese in the ration. It is noticeable that at 8 weeks of age as the quantity of manganese increases

there is a lower average degree of bowing. Lot 1 receiving the basal ration (7 ppm. of manganese) had an average degree of bowing of 3.23 while lot 8 receiving 107 ppm. of manganese had an average of 1.36.

The average weight of the males at 8 weeks of age was heaviest in lot 4 which received 37 ppm. of manganese, while the heaviest average weight of the females was in lot 5 receiving 47 ppm. of manganese. The lowest average weight was that of lot 1 which received only the basal ration which

TABLE 1

Effect of level of manganese intake on growth and incidence of perosis. First experiment[1]

LOT NO.	MANGANESE PPM.	AVERAGE WEIGHT OF CHICKS, GRAMS		AVERAGE DEGREE OF BOWING		PER CENT OF SLIPPED TENDON	MORTALITY PER CENT
		Male	Female	4 weeks	8 weeks		
1	7	433	442	3.0	3.2	77.1	20.0
2	17	575	576	2.9	2.9	54.3	17.1
3	27	641	560	2.5	2.5	25.7	20.0
4	37	693	582	1.8	1.7	0.0	5.7
5	47	678	621	1.7	1.5	0.0	5.7
6	67	619	562	1.8	1.4	2.9	0.0
7	87	627	556	1.7	1.3	0.0	2.9
8	107	658	605	1.7	1.3	0.0	2.9

[1] Thirty-five chicks selected at end of first week for each lot in experiment. All chicks started in lot in which they continued.

contained 7 ppm. of manganese. When larger quantities of manganese were added to the ration there was not a marked difference in average weight at 8 weeks of age. There was, however, a lower average weight in all lots when the quantity of manganese was above 47 ppm.

The percentage of mortality decreased as the quantity of manganese in the ration was increased. Most of the mortality in the lots in which a high percentage of slipped tendon occurred was caused by inability of the chicks so affected to obtain enough feed.

SECOND EXPERIMENT

Nine lots of Rhode Island Red chicks containing thirty chicks each were used in this experiment. The basal all-mash was supplemented by adding manganese sulfate ($MnSO_4 \cdot 2H_2O$) as follows: lot 11, none; lot 12, 10 ppm. of manganese; lot 13, 20 ppm. of manganese; lot 14, 30 ppm. of manganese; lot 15, 40 ppm. of manganese; lot 16, 80 ppm. of manganese; lot 17, 160 ppm. of manganese; lot 18, 320 ppm. of manganese; lot 19, 640 ppm. of manganese. The results of this experiment are shown in table 2.

TABLE 2

Effect of level of manganese intake on growth and incidence of perosis. Second experiment

LOT NO.	MANGANESE PPM.	NUMBER SURVIVING 2 WEEKS	AVERAGE WEIGHT OF CHICKS, GRAMS		AVERAGE DEGREE OF BOWING		PER CENT OF SLIPPED TENDON	MORTALITY PER CENT
			Male	Female	4 weeks	8 weeks		
11	6	24	430	365	3.0	3.1	45.8	36.7
12	16	28	461	428	3.1	3.1	64.3	20.0
13	26	19	584	489	2.7	2.1	21.1	40.0
14	36	27	609	531	1.9	1.7	3.7	10.0
15	46	23	520	543	1.8	1.7	0.0	26.7
16	86	26	616	524	1.8	1.7	0.0	13.3
17	166	28	584	506	1.8	1.4	0.0	10.0
18	326	25	559	568	1.9	1.3	0.0	16.7
19	646	26	629	634	1.7	1.2	0.0	13.3

Since there was a rather high mortality in some lots during the first 2 weeks, the percentage of slipped tendons is based on the number alive at the end of that period. There were no cases of slipping at that time.

The lower quantities of manganese again allowed the incidence of an appreciable percentage of slipped tendons. There was not so large a percentage of slipped tendons in the lot fed the lowest level of manganese as in the first experiment. The degree of bowing of the legs, however, indicates an advanced stage which closely approaches actual slipping of the tendon. The greatest differences in degree of bowing were noted at the eighth week. Degree of bowing was again influenced inversely by the amount of manganese fed the chicks.

This experiment again indicates that at least 30 ppm. of manganese should be added to this basal mash for satisfactory growth of chicks without the incidence of slipped tendon. The quantities of manganese in this experiment which were much higher than in the first trial failed to manifest a toxic effect.

DISCUSSION

In these experiments a minimum of 30 ppm. of manganese added to the basal ration used was necessary for good growth and for the prevention of slipped tendons. These experiments agree in this regard with those reported by Wilgus, Norris and Heuser ('36, '37) and Gallup and Norris ('37 a). The former investigators found that the addition of 0.0025% of manganese to a diet containing 0.0010% of this element was sufficient almost entirely to prevent perosis. The latter investigators observed that occasionally a chick receiving an adequate amount of manganese developed perosis during the first 10 days. The same workers (Gallup and Norris, '37 b) found that the isolated cases developing early in life could not be prevented by adding manganese in high concentrations to the drinking water or to the feed. Results obtained in lot 6 of the first experiment reported herein, in which one chick developed slipped tendons although the mash contained 67 ppm. of manganese, are in agreement with the results reported by Gallup and Norris. The abnormality was apparent at the end of the second week, and the tendon slipped during the fourth week.

Lyons and Insko ('37) fed to laying hens diets which produced a high percentage of slipped tendons in chicks. This treatment greatly reduced hatchability. The embryos which failed to hatch had very short legs, protruding abdomen, 'parrot beak' and bulging head. The addition of manganese to the diet prevented this condition. An experiment just completed indicates that hens on a diet deficient in manganese produce chicks which develop perosis earlier than chicks whose mothers receive a diet containing a satisfactory quantity of manganese.

The experiments reported herein indicate that manganese fed as manganese sulfate ($MnSO_4 \cdot 2H_2O$) at levels as high as 646 ppm. was not toxic. This is in agreement with the work of Gallup and Norris ('37 a) who used several manganese compounds with the same results.

Even though toxic levels of manganese were not reached in these experiments, the results show that there is a wide range between the minimum protective level and the level at which toxic symptoms appear (wide therapeutic index). Heller and Penquite ('37) have shown that a ration containing approximately 4800 ppm. (0.48%) is highly toxic to young growing chicks. The exact minimum protective level of manganese cannot be definitely established until we have a thorough knowledge of the relationship between the calcium and phosphorus content of a ration and the availabity of manganese. There are perhaps three main factors which operate to determine the minimum protective level of manganese in the chick's diet: 1) the level of available manganese in the diet of the mother hen; 2) the calcium and phosphorus content of the ration; and 3) the availability of the manganese supplement. However, as stated above, the minimum protective level of manganese under the conditions of the experiments herein reported appears to be near 35 or 40 ppm. of manganese.

SUMMARY

These experiments were designed to determine the level of manganese most satisfactory for growth and for the prevention of perosis. An all-mash ration containing 6 or 7 ppm. of manganese was supplemented with manganese sulfate.

A minimum of 30 ppm. of manganese added to the basal ration used was necessary for good growth and for the prevention of slipped tendons. Lower quantities allowed the incidence of an appreciable percentage of slipped tendons. The degree of bowing of the legs was inversely proportional to the content of manganese in the feed up to about 30 ppm. As much as 646 ppm. was not toxic.

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SUP

SUPPLEMENT

PROCEEDINGS OF THE FIFTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

SOUTHERN HOTEL, BALTIMORE, MARCH 30, 1938

The fifth annual meeting of the American Institute of Nutrition was held in Baltimore, at the Southern Hotel on March 30, 1938. One hundred and twelve members and 486 guests registered but there were over 600 in attendance during the scientific session.

COUNCIL MEETING

The Council meeting was held at the Southern Hotel, Tuesday evening, March 29th, 6.30 P.M. All members were present except L. A. Maynard. R. M. Bethke, chairman of the nominating committee, John R. Murlin, editor of The Journal of Nutrition and E. M. Nelson, chairman of the committee on vitamin nomenclature were called on for reports in connection with their activities. Formal actions of the Council are reported in the minutes of the general business session.

SCIENTIFIC SESSIONS

President Mary Swartz Rose presided at the general scientific sessions and business meeting. The scientific program was initiated at 9.40 A.M. March 30th, and proceeded on schedule time. All the papers listed on the program were given.

The six discussion groups attracted and held the interest of large audiences. The success of these conferences in satisfying the interest of the membership and friends in current investigation has been most gratifying. The group leaders have been most painstaking and skillful in planning for and promoting well-directed discussions by highly qualified speakers. It is evident that this phase of the program is one of the most attractive features of the annual meeting.

BUSINESS SESSION

The business meeting convened at 11.45 A.M. and was called to order by President Mary Swartz Rose. The reading of the minutes of the preceding meeting was dispensed with since they had been published in The Journal of Nutrition and could be read by all.

In accordance with the by-laws members in good standing who have reached the age of 65 years become emeritus members. It was announced that Thomas B. Cooley and Percy R. Howe have attained this rank of service.

The report of the treasurer was read by George R. Cowgill. The president appointed E. M. Nelson and H. E. Himwich to serve as auditors. They examined the treasurer's books and declared them correct. It was moved by Arthur Knudson and seconded by A. H. Smith that the report of the treasurer be accepted. The motion carried.

The council recommended to the members that the annual dues for 1938–1939 be $1.00. It was so moved by Grace MacLeod and seconded by A. H. Smith. The motion carried.

In order to take care of increasing demands of the annual meeting the council recommended that a registration fee of 50 cents hereafter be charged to defray the expenses of the meeting; the surplus of previous years may be used for this purpose if a deficit occurs. It was so moved by I. McQuarrie and seconded by A. H. Smith. The motion carried.

The council recommended that the following candidates be elected to membership in the American Institute of Nutrition:

Herbert G. Barott	William J. Dann	Thomas H. Jukes
Carl A. Baumann	Henry J. Gerstenberger	Paul H. Phillips
Ernestine Becker	Tom S. Hamilton	Tom D. Spies
Hugh D. Branion	Margaret W. Johnston	

A. R. Rose moved and Arthur Knudson seconded that the secretary cast a unanimous vote for the election to membership of the candidates approved by the council. The motion carried and the secretary declared the candidates duly elected members of the American Institute of Nutrition.

A summary of the questionaire that was prepared and sent to the members to ascertain their wishes on the type of program desired and the place and time of meeting was presented by R. M. Bethke for the committee which included George R. Cowgill and Icie G. Macy. The answers indicated that the large majority of the members desired that the meetings of the American Institute of Nutrition be held in connection with the meetings of the Federation of American Societies for Experimental Biology and that the Institute remain independent for the time being to allow for further development of policies and experimentation with the program. Moreover, the answers indicated that on the whole the members were satisfied with the type of programs that have been suggested by the members and arranged by the council. It was moved by W. M. Sperry and seconded by E. M. Nelson that the 1939 meeting of the American Institute of Nutrition be held in connection with the Federation. The motion carried.

President Rose announced that the committee composed of R. M. Bethke, L. J. Roberts, W. C. Rose, I. G. Macy and herself for setting up standards for nutritionists in public health departments had fulfilled its duties. The report of the committee was approved by the council and forwarded to Martha M. Eliot of the Children's Bureau. It was also announced that the report is on file and copies may be obtained from the secretary by any of the members who desire to see it.

The activities of the committee on vitamin nomenclature were reported by the chairman, E. M. Nelson, for his collaborators, E. V. McCollum and H. C. Sherman. He announced that the Foods Committee and the Council on Pharmacy of the American Medical Association had expressed its willingness to give up the use of cevitamic acid and accepted the use of ascorbic acid. The following report was given:

'Vitamin F'

The term 'Vitamin F' has been used in various ways in the past but recently has come into wide-spread use in promoting the sale of linseed oil and products alleged to contain so-called 'essential fatty acids.' A group of biochemists interested in fat metabolism gave consideration to this matter during our last

meeting. They forwarded to this committee their recommendation that the term 'Vitamin F' should not be used in referring to linoleic or linolenic acids, or so-called 'essential fatty acids.' Your committee is in accord with these views. It is recommended that the term 'Vitamin F' should not be used in referring to linoleic or linolenic acids or any fatty acids or mixtures of fatty acids.

It was moved by E. M. Nelson and seconded by George R. Cowgill that the term 'Vitamin F' should not be used in referring to linoleic or linolenic acids or any fatty acids or mixtures of fatty acids. The motion carried.

Riboflavin

At the last meeting your committee recommended that the term 'riboflavin' be used to designate the compound identified as '6,7 dimethyl-9 (d ribityl) isoalloxazine,' and that the terms 'vitamin B_2' and 'vitamin G' be no longer used. This recommendation was approved by the Society but final adoption was withheld until the opinions of other groups had been ascertained. No definite objections have been received to this proposal and it is therefore recommended that the term 'riboflavin' be formally adopted.

It was moved by E. M. Nelson and seconded by A. H. Smith that the term 'riboflavin' be formally adopted. The motion carried.

Vitamin A

Your committee has agreed upon a plan for choosing a name for Vitamin A to distinguish it from carotenoids of plant origin having Vitamin A activity. Investigators in this country and abroad will be consulted before a definite recommendation is made to the Society.

Editor John R. Murlin reported on the present status of The Journal of Nutrition. He announced the plan for the publication of a general index to The Journal of Nutrition following the completion of the fifteenth volume, the total cost of which would be about $450. It is estimated that the subscription to members will amount to about 60 to 75 cents. The publisher has requested that the American Institute of Nutrition guarantee one-half of the total cost or $225. It was moved by Doctor Murlin that the Institute go on record as approving the guaranty since there was little likelihood that it would be necessary to draw upon the Institute as the present subscription to the Journal would most probably take care of the cost.

After careful consideration the motion was amended by R. Adams Dutcher and seconded by V. C. Myers to leave the manner of meeting the deficit, if any, to the Council with power. Thus amended the motion carried.

Editor Murlin discussed the present status of the Journal in relation to the number of manuscripts sent in to those accepted and the economies being exercised by the new management of The Wistar Institute. The number of pages in a volume is being more rigidly limited to near 600 (the agreed-upon number) by the publishers. Numerous excellent manuscripts must now be rejected because of lack of publication space. In order to meet the present publication needs two possible solutions were considered, namely, reducing the length of the papers accepted or increasing the number of volumes of the Journal per year. Henry C. Sherman and others made a plea for more concise style in the papers that are published. The council recommended that The Journal of Nutrition be increased from two to three volumes per year when the editorial board and publisher think feasible, provided three-fourths of the members approve. It was moved by E. F. DuBois and passed by the Society that the recommendation of the council be accepted.

The council recommended the acceptance of a proposed award of $1000.00 yearly to the American Institute of Nutrition by Mead Johnson and Company for a term of 5 years for meritorious research work on the 'B-complex.' It was moved by R. M. Bethke and seconded by L. C. Norris that the award be accepted and that a committee of five members be appointed by the chair to consider the details of the award. The motion carried. (President McCollum later appointed the following members to serve on the award committee: George R. Cowgill, chairman; L. A. Maynard, A. G. Hogan, I. G. Macy and C. A. Elvehjem.)

In accordance with the constitution President Rose named the following nominating committee for 1939: Victor C. Myers, chairman; I. McQuarrie, A. G. Hogan, Martha M. Eliot and R. C. Lewis.

President Rose asked the tellers, N. B. Guerrant, chairman, Helen A. Hunscher and H. H. Williams, to report on the count of the ballots for the election of officers for 1939. The results were as follows: President, E. V. McCollum; vice-president, T. M. Carpenter; treasurer, George R. Cowgill; secretary, L. A. Maynard; and councillor, Helen S. Mitchell. No new members of the editorial board were elected because no associate member had served the full term of 4 years.

Paul E. Howe moved that the secretary be given a rising vote of thanks for her 'excellent services' during the past 4 years. The Society so responded with enthusiastic applause.[1]

It was moved by Henry C. Sherman and seconded by John R. Murlin that the secretary be instructed to extend heartiest thanks to the local committee and the management of the Southern Hotel for the splendid arrangements provided for the meeting and for the gracious services and courtesies extended to the members of the American Institute of Nutrition and their guests, all of which made the meeting so successful and enjoyable.

LUNCHEON

The noon luncheon in the Banquet Hall of the Southern Hotel was attended by 251 members and guests.

DINNER

A subscription dinner in honor of Prof. Russell H. Chittenden was held in the ball room of the Southern Hotel and was attended by 301 members and their friends. Among the honored guests were William H. Howell, William T. Porter and the presidents of each of the constituent organizations in the Federation of American Societies for Experimental Biology. Pres. Mary Swartz Rose traced in a delightful and entertaining manner the development and activities of the American Institute of Nutrition over its 5 years of existence. John R. Murlin, editor of The Journal of Nutrition served as toastmaster and introduced the honored guest with cogent remarks concerning his activities in the development of

[1] Editorial license.

physiological chemistry and nutrition in this country. Prof. Russell H. Chittenden spoke on "Some Changing Viewpoints in Nutrition."

EDITORIAL BOARD MEETING

The editorial board met at the call of the editor at the Southern Hotel, 6.30 P.M., April 1, 1938. The following members were present: George R. Cowgill, Howard B. Lewis, Arthur H. Smith, Grace MacLeod, Elmer M. Nelson, John B. Brown, William H. Chambers and Albert G. Hogan. The secretary, Icie G. Macy was in attendance to record the minutes of the meeting.

The editor reported on various matters concerning the relations of the journal to The Wistar Institute as publisher. These were, principally, the type of paper used in the journal, a new advertising policy of the Wistar, business arrangements for the general index to be published at the end of the current volume, and the possibility of increasing the number of papers published including expansion to three volumes a year. The board agreed to try for a year requiring authors to condense their material to an average length of not more than ten pages so as to accommodate ten articles in each number. This will be put into effect for all articles accepted after this date (April 1, 1938).

A discussion of policies and criteria for judging manuscripts followed. The associate editors exchanged ideas with the editor and the following criteria for judging manuscripts evolved:

1. Reasons for doing the work and whether the final results have scientific merit.
2. Evidence presented, i.e., adequacy of data.
3. Form and organization.
   a. Use made of illustrative material.
4. Condensation of essential facts.

The 5-year term of John R. Murlin as editor being due to expire on January 1, 1939, the board was asked to consider the question and make some provision for filling the office. He

appointed Icie G. Macy, Secretary of the American Institute of Nutrition, to take the chair and conduct the election and he withdrew from the meeting. The members of the editorial board expressed their deep appreciation for the efficient and outstanding work of Doctor Murlin as editor in building up the Journal. Furthermore, they expressed a genuine desire to have him continue his office as long as it was not too great a burden for him. It was moved by Grace MacLeod and seconded by E. M. Nelson that John R. Murlin be re-elected for a 5-year period as editor of The Journal of Nutrition from January, 1939. The motion carried unanimously. Doctor Murlin accepted the re-appointment on the understanding that he would resign the moment he felt he could not carry on the editorial work efficiently.

There was consideration of the need for an assistant editor to relieve the editor of some of his routine responsibilities. It was moved by Howard B. Lewis and seconded by E. M. Nelson that Doctor Murlin be allowed to select an assistant editor for the coming year. The motion carried.

Respectfully submitted,

Icie G. Macy, *Secretary*
American Institute of Nutrition

ABSTRACTS OF PAPERS

The measurement of the efficiency of diets. New apparatus and procedures. E. B. Forbes, R. W. Swift and Alex Black (by invitation), Institute of Animal Nutrition, Pennsylvania State College.

New equipment was devised and two experimental procedures were demonstrated, with growing albino rats as subjects, for comparing and measuring nutritive values of human diets.

The equipment was a water-sealed respiration chamber permitting normal physical activity of the subjects.

One procedure was a growth experiment, with carbon and nitrogen balances, at intervals, for observing the effects of the progressively increasing severity of the nutritive conditions imposed by a constant food intake, without allowance for the growth of the subjects.

This procedure yielded results in terms of gross efficiency, covering requirements for maintenance and growth together.

The second procedure consisted of nutritive measurements, also by means of balances of carbon and nitrogen, but in terms of net energy and protein.

Balances were determined during fast, at the maintenance level, and at a higher plane, and values were determined for metabolizable energy, heat increment, and net energy—separately for maintenance and growth.

Six human diets, differing in significant ways, were compounded to contain the same gross energy and protein contents. Two of these were compared by the constant food intake method, and all six by the net energy procedure.

The fat composition of milk fed rats. Herbert E. Longenecker (introduced by R. A. Dutcher), Department of Industrial Chemistry, University of Liverpool, England.

This constitutes a detailed examination of the neutral fat fatty acids from rats which were fed milk only for 11 weeks after weaning. An electrically heated and packed fractional distillation apparatus[1] of high efficiency was used for the separation of fatty acid ester mixtures. The analyses indicated 1) that fatty acids lower than C_{10} were not stored in detectable amounts and only 1.4 mols.% of the mixed rat fatty acids consisted of C_{10} and C_{12} acids whereas similar analyses of milk fat[2] show about 23 mols.% of C_4, C_6, C_8, C_{10} and C_{12} acids, 2) a somewhat higher myristic acid content than is usual for rat fat which may be related to the quantity of this acid in milk fat as a direct example of the deposition of a food fatty acid, 3) the presence in this rat fat of tetradecenoic acid, not previously reported, which may be either a normally expected component or one contributed by the milk fat, and 4) that on a relatively low fat diet, the rat apparently constructs a body fat of nearly constant composition containing oleic (45 to 50 mols.%) and palmitic (25 mols.%) acids with smaller amounts (3 to 6 mols.%) of myristic, stearic, hexadecenoic and octadecadienoic acids, and traces of C_{20} saturated and unsaturated acids.

[1] Longenecker, H. E., J. Soc. Chem. Ind., vol. 56, p. 199T (1937).

[2] Hilditch, T. P., and H. E. Longenecker, J. Biol. Chem., vol. 122, p. 497 (1938).

Nicotinic acid in the prevention of blacktongue of dogs. W. H. Sebrell, R. H. Onstott (by invitation), H. F. Fraser (by invitation) and F. S. Daft (by invitation), National Institute of Health.

Five dogs were given a blacktongue producing diet with the addition of nicotinic acid in amounts varying from 1 to 50 mg. biweekly. No blacktongue occurred during the 6-month experimental period, except in the animal receiving 1 mg. One animal died, possibly of riboflavin deficiency. A weekly supplement of 0.5 mg. of riboflavin was then given to each dog.

Five dogs developed blacktongue on the same diet without nicotinic acid. These animals were treated with varying amounts of nicotinic acid until the acute symptoms subsided. They were then maintained in good condition to the end of the experiment on 10 mg. biweekly.

Seven dogs developed blacktongue on the same diet without nicotinic acid and repeated attacks of blacktongue were successfully treated with varying doses of nicotinic acid, which was discontinued when the acute symptoms of each attack subsided.

Synthetic and commercial nicotinic acid were used.

The relation of the vitamin B complex to fat metabolism. E. G. Gavin (by invitation) and E. W. McHenry, School of Hygiene, University of Toronto.

Previous work by Whipple and Church and by us has shown that thiamin causes a formation of fat from carbohydrates in rats; this synthesized fat accumulates in the liver when the diet lacks choline and in the depots if the diet contains ample choline. Supplee and others have reported that rice polish concentrate, used as a source of vitamin B_6, augments the effects of thiamin and flavin in increasing the weight of young rats. The present work indicates that this increase in weight is partially due to a marked effect of the concentrate on body fat, an effect which is not due to the amount of choline in the concentrate. Choline, flavin and thiamin either separately or in several combinations do not give this effect. It is suggested that at least part of the supplementary effect of several members of the vitamin B complex on increases in body weight is due to their relation to fat metabolism. Thiamin causes an accumulation of dietary or synthesized fat in the liver. Vitamin B_6, or another component of rice polish concentrate, promotes a storage of fat in the depots. The effect of rice polish concentrate is augmented by flavin.

Variations in the reproductive behavior of different species of mammals restricted to vitamin E-deficient rations. Byron H. Thomas, C. Y. Cannon (by invitation), S. H. McNutt (by invitation) and Gravers Underbjerg (by invitation), Animal Chemistry and Nutrition, Iowa State College.

Whether rations deficient in vitamin E will interfere with the normal course of reproduction in farm livestock as they do when fed to rats has not been determined heretofore.

We have restricted goats, sheep and rabbits during one or more generations to specially prepared feed mixtures which repeatedly have produced resorption gestations in female rats. The vitamin E in these mixtures of ground grains and finely chopped alfalfa hay was destroyed or inactivated by treatment with an ether solution of ferric chloride and subsequently aging them until after they had acquired a distinctly rancid odor. The mixtures were fortified at feeding time

with a vitamin E-free supplemental mixture compounded primarily to protect against possible other vitamin deficiencies.

Male and female goats were able to reproduce through several generations apparently unhampered when restricted to vitamin E-deficient rations which regularly produced resorption gestations when fed to female rats. Results of a preliminary investigation of 2 years duration involving male and female rabbits agree in part with those yielded by the goats. A number of young lambs have been limited to similar feed mixtures for 1 and 2 years. All have been bred for spring lambs. Some already are exhibiting unmistakable signs of advanced pregnancy. The studies involving rabbits and sheep are being continued.

The foregoing observations appear to have important scientific and practical implications.

Effects of ascorbic acid upon resistance to bacterial toxins. C. G. King, A. Sigal (by invitation) and R. R. Musulin (by invitation), Chemistry Department, University of Pittsburgh.

The protection afforded by different levels of vitamin C intake has been studied in relation to tissue injury and physiologic impairment caused by injections of bacterial toxins. Guinea pigs were fed a modified Sherman diet, supplemented by 0.5 to 5.0 mg. of synthetic ascorbic acid per day. An intake of 0.5 mg. of vitamin per day permits nearly normal growth and affords protection from gross signs of scurvy, but animals receiving 5.0 mg. of vitamin per day were less sensitive to injury by bacterial toxins than were animals receiving only 0.5 mg. per day. The teeth provide a particularly good index to the correlation between nutritional level and tissue injury. Studies of blood cellular constituents, arterial lesions, sugar tolerance, length of survival, and changes in body weight provide less sensitive but confirmatory evidence of the benefit that may be derived from a generous level of ascorbic acid intake in comparison with a level that is barely protective against scurvy. The differences in degree of injury that can be correlated with differences in nutritional level have been found to vary greatly with different toxins. Diphtheria toxin has proved to be the most satisfactory of those studied.

Distribution of vitamin K in biologic material and its probable physiologic significance. Hugh R. Butt, Arnold E. Osterberg (introduced by R. M. Wilder), Mayo Clinic, Rochester, Minn.

A few years ago, Dam discovered a sterol substance in pig liver fat (vitamin K), which would prevent the occurrence of a hemorrhagic diathesis in chicks fed a deficient diet. It was postulated that the absence of this sterol might be indirectly related to the decreased level of prothrombin observed in the circulating blood of these bleeding animals. In individuals with obstructive jaundice there is likewise an apparent decreased quantity of prothrombin in the circulating blood, and in these individuals a hemorrhagic diathesis is not infrequent. The possibility of a deficiency or inadequate absorption of Dam's sterol substance occurring in individuals with obstructive jaundice is considered and the method for preparing this sterol substance from fish meal is described. Its effect upon the prothrombin level of the blood of individuals with obstructive jaundice when administered alone or with bile is discussed, and its probable distribution in biologic material is mentioned.

Some pathological and bacteriological effects of chronic vitamin A deficiency. Aline Underhill Orten (by invitation), Caspar G. Burn (by invitation) and Arthur H. Smith, Departments of Physiological Chemistry and Pathology, Yale University.

A mild chronic or incipient vitamin A deficiency was produced in young rats and the condition maintained for periods up to 1 year. The quantities of vitamin A (I. U.) required to bring about this state have been determined and their relation to age, weight and sex studied. The animals were examined daily and at appropriate intervals groups were sacrificed in order to study the progressive development of pathological and bacteriological changes. The findings have been compared with those from normal control rats for the same age and with those from young animals showing the classical symptoms of advanced vitamin A deficiency.

Slightly subnormal growth, respiratory infections and a relatively high mortality (54%) occurred. The incisor teeth showed progressively loss of pigmentation, development of an opacity, distortion and eventual exfoliation of the erupted portion. Large, non-sensitive growths developed in the maxillae. Histological changes occurred in the incisor teeth similar to those found in the typical, acute A-deficient animals and, in addition, tumor growths (odontomas) and supernumerary teeth had developed. Bacteriological changes were also observed in the chronic A-deficient animals. A shift from the saprophitic flora normally found on the various lining epithelia to potentially pathogenic forms, and eventual invasion of the viscera by these organisms occurred.

The retardation of senescence. C. M. McCay, L. A. Maynard, Gladys Sperling (by invitation), Animal Nutrition Laboratory, Cornell University.

New experiments have been completed to determine the effect of different periods of retardation of growth upon the development of the characteristics of old age and upon the length of the total life span. At the time of weaning white rats were divided into five groups. The first group which represented the control was allowed to grow to maturity at a normal rate. The remainder of the groups was retarded by calorie restriction for periods of 300, 500, 700 and 1000 days, respectively, and then allowed to complete their growth.

An intensive study has been made of these individuals to determine such factors as the growth of the bones, the degree of calcification of the arteries and the cause of death. In the present report attention will be centered upon the retardation of senescence that resulted from slowing the growth rate.

Although the 'normal' growth group originally contained a third of all the animals, the last of this group died at the age of 964 days, leaving animals alive in all the retarded groups. Photographs were used to show the contrast between the 'old' members of the normal growth group and the retarded ones. At the age of 1068 days 12% of the retarded animals were still alive in spite of losing approximately half of them from accidents in the first year of the experiment.

These data indicate that the retardation of growth affords a method of retarding senescence and extending the span of life far beyond the normal. The method provides an effective technic for attacking the problems of aging within the body of experimental animals.

Total energy metabolism of rats at various ages as affected by protein and exercise. A. Black (by invitation) and J. R. Murlin, Department of Vital Economics, University of Rochester.

The total energy metabolism of ninety-six albino rats, comprising six groups of equal number, was studied intermittently for 8½ months.

The protein fed these animals was supplied by whole milk powder and dried whole egg. There were three groups of animals on each protein treatment, two of which received a ration containing 20% protein and one 8% protein. One of the groups given high protein from milk and one from eggs were exercised a definite amount daily. The three groups of animals on a particular protein treatment were fed according to the paired-feeding method as modified to match three animals instead of two. The total energy consumption of all six groups was essentially the same.

The animals, which received their protein from eggs, made greater gains in live weight than was noted with milk. The greatest difference occurred on the 8% protein level and the least difference when exercise was imposed.

The heat production, as measured with a new automatic calorimeter, of the three groups receiving milk was the same on a unit basis showing a decrease with age until the last month when an increase occurred. The exercised group on eggs produced more heat than the two groups which were not exercised. The delayed increased heat production occurred earlier in these groups than in the milk fed animals.

Basal metabolism tended to be slightly higher in the exercised animals.

Effects of prolonged use of extremely low fat diet on adult human subject. W. R. Brown (by invitation), A. E. Hansen (by invitation), G. O. Burr and I. McQuarrie, Departments of Pediatrics and Botany, University of Minnesota.

As a sequel to studies, which suggested an etiological relationship between infantile eczema and a disturbance in the unsaturated fatty acid metabolism, the present investigation was undertaken to determine whether or not the human subject requires preformed fat in the diet, as the young rat does. In the absence of a justifiable opportunity to make such tests on the growing infant, a normal human adult was observed over a period of 6 months while subsisting on a diet practically devoid of fat. Twelve young rats placed on this experimental diet developed typical signs of the fat deficiency disease described by Burr and Burr.

While careful weekly examinations of the experimental subject revealed no clinical evidence of disease as a result of the diet, he manifested certain changes in metabolism toward the end of this period, which are characteristic of the fat-deficiency syndrome as it occurs in rats. The R.Q. following a meal of the diet rose to 1.14 at the end of the period, whereas the maximum at the beginning was 0.99. The iodine number of the serum fatty acids fell from an average of 122 to 92. Direct determinations of the linolic and arachidonic acids of the serum showed them both to decrease approximately 30% during the experimental period. From these findings it is concluded that the human subject, like the rat, requires preformed unsaturated fatty acids in the diet.

Influence of Ca: P ratio of diet on ratio in serum during development and cure of low phosphorus rickets. Henry J. Gerstenberger (introduced by Victor C. Myers), Laboratory of Babies and Childrens Hospital and Department of Pediatrics, School of Medicine, Western Reserve University.

This report presents part of a comprehensive study of rickets in monkeys (Macacus rhesus). Work previously completed has shown that a low phosphorus type of rickets develops regularly and rather easily in monkeys in a sunlight free environment, just as in full-term human infants, even though the diet fed the monkeys has a low calcium, high phosphorus ratio (0.49).

The present report deals with the response of monkeys fed rickets-producing diets, a) with a Ca: P ratio of 0.49, the original diet, and b) with a ratio higher in calcium, more nearly approaching that of cow's milk, 1.3, to treatment with ultraviolet light.

The entire group included twenty-seven monkeys; fourteen were exposed six times weekly to ½ or 1 M.P.E. doses of ultraviolet light and the remainder served as controls.

Of the fourteen monkeys exposed, six had been fed since admission the diet with Ca: P ratio 1.3 and eight the diet with Ca: P ratio 0.49. Progress and kind of healing were checked by determinations of calcium and phosphorus in the blood serum and by the degree of healing of the bones as shown by roentgenograms.

It was found that, under the conditions chosen, the higher Ca: P ratio of the diet used was of greater influence in preventing the drop in the calcium level of the blood serum than was the higher dosage of ultraviolet light.

The role of manganese in bone development. L. C. Norris, W. D. Gallup (by invitation) and C. D. Caskey (by invitation), Department of Poultry Husbandry, Cornell University.

In studies on the role of manganese in nutrition it has been found that manganese is essential for normal bone development in the chick. The tibiae of day-old chicks from hens fed a low-manganese diet (13 p.p.m.) were found to be 12.5 to 13.1% shorter than those of chicks from hens fed a high-manganese diet (200 p.p.m.) and the metatarsi were found to be 14.2 to 15.1% shorter.

The long bones of the legs and wings of chicks fed a low-manganese diet (5.5 p.p.m.) were found at 3 weeks of age to be shorter than those of comparable chicks fed a high-manganese diet (100 p.p.m.). By comparing the femurs of paired chicks it was found that the odds against the shortening of the femurs of the low-manganese chicks being due to chance were 713 to 1 at 3 weeks of age and 3332 to 1 at 4 weeks of age.

This phenomenon is not caused by a disturbance in gross body composition, as the percentage of water, fat and ash of the bodies of paired chicks was essentially the same. The gross composition of the femurs of chicks 3 weeks of age was also approximately the same. However, the manganese content of the leg bones of the low-manganese chicks was less than that of the high-manganese chicks.

ABSTRACTS OF PAPERS READ BY TITLE

On the fatty acids essential in nutrition. G. O. Burr, J. P. Kass (by invitation), J. B. Brown and Jerome Frankel (by invitation), Departments of Botany, University of Minnesota, and Physiological Chemistry, Ohio State University.

Oils can be biologically assayed for their content of essential fatty acids. Although such an assay puts them in an order of curative power corresponding in general with their unsaturation and linoleic acid content as reported in the literature, there are outstanding exceptions. Corn oil is superior to linseed oil. Purified oleic acid from several sources continues to give negative results.

Linoleic acid prepared by debromination of the tetrabromides has a curative value similar to that in the original oil. On the other hand, the so-called β-acid which gives no insoluble tetrabromide in petroleum ether seems to be much less efficient than the α-acid.

Arachidonic acid is also of high curative value. The isomeric fatty acids and their nutritive values are discussed.

Growth of suckling rats in relation to the diet of the mother. Gerald J. Cox, Sara F. Dixon (by invitation), Margaret C. Matuschak (by invitation) and W. Edgar Walker (by invitation), Nutrition Fellowship, Mellon Institute.

The individual weights of 508 suckling rats in seven groups were determined daily from birth to weaning at 21 days. The mean weights at weaning varied from 31.9 gm. on a cornmeal-milk powder diet to 50.3 on a meat-calcium carbonate ration.

Conclusions were as follows: 1) The rate of growth of suckling rats is a function of the diet of the mother. 2) The coefficient of variation from all rats was about 10% at birth, a maximum of about 16% at 10 days and improved to 12% at weaning. 3) The better the diet of the mother, the lower the coefficient of variation. 4) Growth rate declines continuously after the fourth day. 5) There is no evidence of abrupt change of growth rate. 6) Weaning weight is positively correlated with birth weight.

Groups A and B of six rats each were suckled successively by three mothers for 8-hour periods so that group A was always suckled by a mother rested for 8 hours and group B suckled the next period after group A. The Anderson and Smith diet was used. Eighteen group A rats averaged 67.3 gm. at 21 days; eighteen group B rats averaged 47.7.

Comparative mineral balance studies with children receiving two levels of pasteurized and evaporated milk in high and low cereal diets. Amy L. Daniels, Iowa Child Welfare Research Station, University of Iowa.

The substitution of superheated milk, more particularly evaporated, for raw or pasteurized in the human dietary, has led to a study of its nutritive value by comparing the calcium, phosphorus and nitrogen retentions and fecal excretions of nineteen preschool children receiving during two successive metabolism periods the same diets with comparable amounts of pasteurized and evaporated milk. The vitamin D intake was the same in all cases; and the vitamin C was adequate. High and low cereal diets were tested with two levels of milk ingestion.

Some clinical studies on vitamin C deficiency. William Freeman and W. Everett Glass (introduced by M. O. Lee), Worcester State Hospital, Worcester, Mass.

A sample of a mental hospital population showed that 53% of the psychotic patients and 28% of the healthy, normal employees had a subnormal fasting blood vitamin C level. None of the subjects showed any signs of scurvy.

No correlation was found between the ascorbic acid blood level and the age, sex, psychotic state, length of hospitalization, nutritional index, rectal temperature, systolic blood pressure, pulse pressure and blood chemical and morphological elements. Neither were there any correlations found between the blood vitamin C level and the incidence of bleeding gums, gingivitis, and the number of capillary skin hemorrhages.

Central autolysis of the adrenal glands at autopsy was found to be correlated with a subnormal antemortem ascorbic acid level of the blood.

The ascorbic acid content of various organs at autopsy showed a wide range of variation and was not parallel with the post mortem blood level. When a deficient level was obtained, the ascorbic acid content of the adrenal glands was the lowest of any organ, and when an adequate level obtained, the adrenal glands had the highest concentration.

Bringing the blood ascorbic acid from a subnormal point to one well above the minimum normal level required varying amounts, between patients, of daily intravenous administration of vitamin C. To maintain the blood at a constant, normal level also required varying amounts, between patients, of daily intravenous administration.

The influence of calcium and phosphorus on iron assimilation. S. W. Kletzien, State Institute for the Study of Malignant Disease, Buffalo, N. Y.

In previous communications it has been shown that various inorganic salts of calcium depress the normal assimilation of iron in the young growing rat, and that calcium carbonate in the amounts fed interfered with the maternal and foetal storage of iron. It is now found that the citrate, tartrate, and acetate of calcium do likewise, and that the malate and oxalate do so to a lesser degree.

As regards phosphorus, the surmise exists that it in some way interferes with maximum iron assimilation. In the light of our findings this is not substantiated. Animals (rats) receiving phosphoric acid as such assimilated more iron than control animals receiving an equivalent amount of phosphorus as tri-calcium phosphate. Lower iron values are obtained only with the simultaneous introduction of calcium. In regard to calcium/phosphorus ratios, evidence is accumulating which supports the belief that a low Ca/P ratio improves iron assimilation appreciably.

The vitamin B retentions of infants. Elizabeth Knott (by invitation) and Frederic W. Schlutz, Department of Pediatrics, The University of Chicago.

Retentions of vitamin B_1 have been investigated for four infants during a series of controlled metabolism periods. Each infant has been studied for at least ten periods of 5 days duration each. Urine, feces, and food have been analyzed for their vitamin B_1 content by means of carefully controlled biological assay.

By altering the level of vitamin B_1 ingestion, the optimum intake has been determined which will give maximum retention of the vitamin.

In comparing results with those previously reported for preschool children, it is noted that a higher requirement of vitamin B_1 is needed per unit of body weight for infants than for the older children. With the infants, the vitamin is almost completely absorbed as contrasted to the considerable quantities excreted in the feces of older children. Urinary excretion of vitamin B_1, in both infants and children, increases with increased intakes, but at a slower rate than the intake if the child has been receiving suboptimal amounts. If optimum or above optimum amounts are ingested, urinary excretion increases until a balance is established which seems to suit the child's required needs.

Effect of topical application of cod liver oil on wound healing and on liver vitamin A in normal and chronically A-deficient rats. Elaine P. Ralli and Harold Brandaleone (by invitation), Laboratories of Department of Medicine, New York University College of Medicine.

Groups of rats rendered chronically deficient in vitamin A were wounded under aseptic conditions at the time the symptoms of vitamin A deficiency appeared. In one group of animals the wounds were treated with weighed amounts of a standard cod liver oil; in the other group of rats no cod liver oil was applied to the wound. Groups of normal rats were similarly wounded. The rate of healing in each group was determined by measuring the wounds daily and by noting the time at which healing occurred. In all groups at the time the animals were placed on the A-deficient diet a certain number of animals were sacrificed and the vitamin A content of the livers determined. This was done again at the time the symptoms of vitamin A deficiency appeared, and at the end of the experiment the vitamin A content of the livers was determined. It was found that when the wounds in the A-deficient animals were treated with cod liver oil the rate of healing was twice as fast as in the untreated wound, but the vitamin A content of the liver in the group whose wounds were treated with cod liver oil was not higher. It is suggested that cod liver oil has a specific effect on wound healing not necessarily related to vitamin A.

Weights of diabetic organs in relation to a possible pituitary influence. Howard F. Root, George F. Baker Clinic, New England Deaconess Hospital, Boston, Mass.

Since the demonstration that it is possible to produce permanent experimental diabetes in the dog by injection of an extract of the anterior pituitary lobe of oxen, it is important to investigate the possibility that some diabetogenic hormone is a factor in the causation of many if not all cases of diabetes. The high incidence of diabetes in acromegaly clearly indicates that a diabetogenic factor is present in these cases in association with whatever factor it is which produces the great increase in size not only of the skeleton but of the internal organs. If such a diabetogenic factor is an important agent in producing clinical diabetes one might expect some correlation between the size of the pancreas and the size of other organs. In acromegaly the pancreas as well as other tissues is often enlarged and by contrast, in Simmon's disease the pancreas as well as other

organs shows atrophy. In analyzing 200 diabetic autopsies, studies of tissue weights of those cases in which the pancreas was notably small was made in comparison with cases in which the pancreas was notably large. A study of the relationships in comparison with acromegalic diabetes is presented.

Cost and nutritive value of American diets. Hazel K. Stiebeling, Bureau of Home Economics, U. S. Department of Agriculture.

As part of a nation wide study of family consumption conducted by federal agencies, dietary information from about 25,000 non-relief families has been analyzed after classification by region, degree of urbanization, color of family, level of expenditure for food, and to some extent also by season, occupation and the composition of the family.

A comparison of the nutritive value of the food received in the kitchen during 1-week periods with specifications for 'good diets' essentially the same as those proposed by the League of Nation's Technical Commission on Nutrition indicates that with present day food habits and 1936 prices families require more than $90 per person per year to buy 'good diets,' some require as much as $250. Expenditures for food by the interquartile group fell between approximately $100 and $160 per person per year. The dietary deficiency most frequently encountered was calcium. The highest proportion of 'good diets' was found on the Pacific coast where the consumption of milk, fruits and the succulent vegetables was highest for any section of the country, and the lowest in the southeast where sugars and fats tend to displace these protective foods. As a rule farm family diets were found to be better than those of city families; white family diets better than Negro, and diets of families including young children better than those composed only of adults.

Cellular changes in the anterior hypophyses of vitamin A deficient rats. T. S. Sutton and B. J. Brief (by invitation), Dairy Industries, Ohio Agriculture Experiment Station and Department of Anatomy, Ohio State University.

Differential cell counts were made on anterior hypophyses from normal, vitamin A deficient, and castrate rats, in an attempt to determine the 'modus operandi' of vitamin A deficiency in causing sterility. It was found that a deficiency of vitamin A caused an increase in the percentage of beta (basophilic) cells, together with an increase in the number of beta cells containing a macula. This increase in beta cells was significant in vitamin A deficient animals of both sexes. However, when compared to normals, a greater relative increase in beta cells was noted in hypophyses from vitamin A deficient males than from vitamin A deficient females. The changes noted in vitamin A deficiency approach those found in true castrates. The greater relative change in vitamin A deficient males, when compared to similar vitamin A deficient females, may be accounted for on the basis of a more nearly complete destruction of the germinal epithelium of the male gonad.

This study presents further evidence that a vitamin A deficient diet causes direct damage to the gonad, the increase of beta cells representing a compensatory change in the hypophysis.

Toxemic pregnancy in the rat. Pearl P. Swanson and P. Mabel Nelson, Nutrition Laboratory, Food and Nutrition Department, Iowa State College.

If rats are fed a supposedly adequate diet containing dried canned pork muscle as its protein constituent, as many as 30% of the females may die in parturition, exhibiting many signs of toxemia. Symptoms develop suddenly; muscle tone disappears, body temperature falls, the rat trembles, respiration becomes rapid and labored, bloody urine is excreted. Severe convulsions with drooling from the nose and mouth follow. Autopsy shows an extremely yellow and friable liver with congestion, hemorrhage, or degeneration of the kidney. A reddish serous fluid surrounds the lungs. The pancreas is embedded in a jelly-like mucous. The blood is watery and does not clot. Histologic studies are being made, as well as chemical determinations of the glycogen and fat content of the liver.

Two grams of fresh liver as a daily dietary supplement protected ten rats through four successive pregnancies. Choline and lecithin, tested as possible potent factors in liver, aggravated the occurrence of the syndrome as did liver extract. However, lipocaic, isolated from pancreas by Dragstedt at a daily dosage of only 4 mg. was fully protective in forty litterings.

The fatty appearance of the liver suggests a deranged fat metabolism. If a pancreatic hormone is concerned with fat metabolism, apparently the diet fed does not provide precursors for the elaboration of sufficient lipocaic to meet the needs of pregnancy.

GROUP DISCUSSIONS

3 to 5 P.M., March 30th

1. The use and place of the chick in nutritional studies. R. M. Bethke, presiding.

The chick has a definite place as an experimental subject a) in the investigation of the nutrition of the species itself and b) for the study and assay of various nutritive factors. It has already proved valuable in the differentiation of the several antirachitic factors, in the assay of vitamin K (anti-hemorrhagic factor) and in blood regeneration studies. One method for the assay of antirachitic potency specifies the use of the chick. The study of embryos from the eggs of hens on a low vitamin E ration has revealed information concerning rapid cell proliferation. The chick has been used extensively in the study and differentiation of the members of the vitamin 'B and G complex.' Also in the study of the nutritive value of proteins it has been of importance. Our increased knowledge of the nutritive requirements of the species has permitted the formulation of more satisfactory synthetic rations.

Success in the use of the chick in the laboratory is determined by genetic factors, freedom from disease, selection as to age, sex, nutritional history of the hens, hours of light, temperature and humidity, physical properties of the feed, training it to eat and the method of distribution in experimental pens.

2. The significance of dietary fat. J. B. Brown, presiding.

Following some introductory remarks by the chairman, the subject of dietary fat as affecting digestion and absorption was discussed by J. P. Quigley. Comments and questions by members of the group were directed largely at the question of the possible effects of ingested fats on the absorption of other food constituents.

G. O. Burr then described the more recent findings on the essential nature of fat. He pointed out that no one fatty acid can be regarded as the essential one but rather that several serve; however, the responses to the administration of the individual acids vary in rapidity and degree of effectiveness.

The practical value of dietary fat in the treatment of disease was discussed by I. McQuarrie largely from the standpoint of the uses of low and high fat diets. Comments by other workers in the field showed the wide applicability of controlled diets and the wide field for further clinical study.

W. M. Sperry then presented the problem of optimum fat intake in man. He pointed out that both quality and quantity are concerned. The quality is probably of less practical importance than the quantity because of the complex nature of most natural fats and of the characteristics they have in common. Experimental work has shown that a high fat diet is less conducive to sustained muscular effort than a high sugar diet yet no exact standards of optimum intake are as yet available.

The discussion closed with some observations on the possible toxic effects of dietary fats. Approximately 110 members and guests attended the meeting of whom approximately fifteen joined in the discussion led by the listed speakers.

3. Members of the vitamin B complex. C. A. Elvehjem, presiding.

A mimeographed outline of the factors included in the vitamin B complex prepared by the chairman was passed out to all those present. W. H. Sebrell opened the program with a movie on the production and cure of blacktongue in dogs. This was followed by a report from Tom Spies on the successful use of nicotinic acid in the treatment of a large number of cases of pellagra. There was a great deal of interest in the colored photographs showing the dramatic responses obtained after the administration of nicotinic acid. After these papers there was considerable discussion on the subject of pellagra and nicotinic acid.

Paul György continued with a brief but complete survey on vitamin B_6. He especially emphasized the recent work on the isolation of vitamin B_6 in crystalline form. This paper also led to a number of questions on the relation of other factors such as fat and flavin to vitamin B_6 deficiency. W. J. Dann outlined the work which has been done on the filtrate factor, specifying that the filtrate factor referred to the chick antidermatitis factor. He pointed out that very few laboratories were working on the filtrate factor and that undoubtedly the so-called filtrate contains several rather than one factor. E. M. Nelson concluded by referring in a general way to the accepted nomenclature for the B factors and dealt specifically with the possibility of a more specific name for the chick antidermatitis factor. After his paper there was still considerable discussion on the entire subject. About 275 attended this group discussion.

4. Methods of judging nutritional status and requirements. Lydia J. Roberts, presiding.

Great interest was shown in the question "Does the Biophotometer Measure the Vitamin A Stores?" The problems involved in the validity of the test and possibility of a straight line relation of the readings to vitamin A stores were discussed. Controversy was raised concerning reproducibility of data, learning factor, standard units for recording means of converting findings in universal terms, standards for adults and children and the effect of supplementing the diet with vitamin A. The necessity for consideration of visual acuity, method of controlling light, standard position for most sensitive portion of the eye and calibrations of instrument were stressed. So many points of view were expressed that it is difficult to state conclusions except that the principle of the test is valuable in sorting out the individuals with subclinical deficiencies but for precise determination of degree of subnormal vitamin A nutrition standardization of method in terms of the physiology of the eye needs further investigation. Many people took part in the discussion which was led by workers from the U. S. Public Health Service, The University of Chicago and the Bureau of Home Economics, U. S. Department of Agriculture.

The topic "The Urinary Excretion of Ascorbic Acid as a Measure of the Body's Store and Requirement of This Vitamin" was introduced by H. M. Hauck. The method can be used to determine the amount needed to produce saturation of the body whereas random measurement does not indicate the adequacy of the diet. Large test doses administered after saturation of the body give reliable data on requirement. Twenty-five milligrams for 6 successive days followed by a test dose of 400 mg. were found inadequate for saturation, whereas,

75 mg. daily were adequate. Factors other than diet to be considered in using these methods are environmental temperature, effect of drugs, reaction of diet and non-specific reducing substances in the urine.

Estelle E. Hawley opened the question of "Reduced Blood Ascorbic Acid as a Measure of Vitamin C Stores." The level in the blood is a valid indication of the state of vitamin C stores. The range of data rather than absolute values must be considered. Concentrations below 0.5 mg. per 100 cc. indicates scurvy; 0.5 to 0.8 mg., low normal; 0.8 to 1.0 mg., adequate, and 1 or more, optimal vitamin C nutrition. Amounts of ascorbic acid in the diet were related to the levels found in the blood.

5. Mineral elements in nutrition. Henry C. Sherman, presiding.

E. V. McCollum stated that the older work indicated that Na, Cl, K, P, Ca, and Fe are essential. The modern era begins with Baumann's discovery of I in thyroid, and now Cu, Mn, Zn, Mg, and Co are added to the indispensable mineral elements. The question regarding essential nature of Br and B is not settled; Al and F seem unnecessary whereas the status of As, Se, Rb, Va and Ni is not settled. Considerable time was spent discussing the newer features of the deficiency of Mg, Mn, and Co.

A. H. Smith confined his discussion to the recent contributions which showed the influence of mineral nutrition on the blood. The present status of milk anemia and the significance of Fe and Cu was emphasized. The importance of normal gastro-intestinal function on the utilization of Fe as shown by hematopoiesis was emphasized. The effect of a deficiency of Mn, Mg and Co on the blood picture was discussed. The significance of mineral nutrition in maintaining a normal state in the blood was emphasized both from the point of view of scientific interest and of practical importance.

Genevieve Stearns pointed out the uncertainty in depending on mineral analyses of the skeleton in the literature. The proportion of water and of minerals are inversely proportional in the skeleton just as in the body as a whole. There are 1500 to 2500 gm. of Ca in the adult skeleton, the rate of growth influencing this. The essential proportionality between intake and retention of calcium by young children was emphasized as was the dependence upon vitamin D by children in several age groups. Need for Ca is greatest during lactation when 1.5 gm. per day with vitamin D is indicated. A minimum of 1 gm. per day with vitamin D is recommended for children. The enhanced retention of Ca with vitamin D is not to be taken as reducing the amount of Ca necessary in the diet.

E. J. Underwood, of New Zealand, discussed the minimum requirement of Co at length indicating that for calves 0.1 mg. per day was enough. In response to a question by A. H. Smith, Genevieve Stearns indicated a superiority of the water-soluble preparations of vitamin D. W. D. Armstrong also made comments.

6. The relation of vitamins to clinical conditions. Frederick F. Tisdall, presiding.

Vitamin A

C. D. May: Cornified epithelial cells in areas where such do not ordinarily occur means a vitamin A deficiency. A very severe deficiency over a long period of time is necessary to produce this cornification.

Recently many people have been attempting to measure light adaptation and using this as a means of measuring vitamin A deficiency. This test involves a moderate degree of intelligence in and a great deal of application by the subject. Other factors besides vitamin A have to do with light adaptation. There is a quantitative relation between the amount of vitamin A and the blue color produced in the Carr Price reaction. For the past 2 years we have been using the Evelyn photoelectric colorimeter for measuring the color produced in this reaction.

Vitamin C

K. Evelyn demonstrated the use of the photoelectric colorimeter devised by him in the measurement of ascorbic acid. By the use of this instrument, consisting essentially of suitable filters, a photo-electric cell and a galvanometer, the reduction of the indo-phenol reagent can be rapidly and accurately determined when an ascorbic acid solution is added to an excess of the dye. The effect of interfering substances upon the rate and amount of reduction was discussed.

W. H. Eddy pointed out that there was a good deal of interpretation to do in vitamin C work even after precision measurements had been made by an instrument such as Doctor Evelyn's. In his own work he pays no attention to single estimations on the ascorbic acid content of the blood, but administers 500 mg. of ascorbic acid by mouth and then by hourly measurements of the ascorbic acid content of urine and blood is able to obtain information concerning the state of nutrition with regard to vitamin C. He found that per unit of size California and Florida oranges were equally valuable in ascorbic acid. While the one had a higher ascorbic acid content per unit of juice, the other yielded more juice. During storage there is little loss of vitamin C in oranges, and a large loss in apples. Spinach stored at room temperature will lose all its vitamin C in a few days. Unrefrigerated handling of fresh fruits and vegetables in the market brings about large losses in vitamin C.

C. G. King stated that large numbers of the population at large are suffering from a deficiency of vitamin C. Where vitamins such as C and D must be administered, we cannot depend upon the diet. Vitamin C is easily destroyed and vitamin D is not widely distributed. In 50% of cases of scurvy, investigation proved that the mother had lied about the administration of vitamin C. The ascorbic acid content of the body tissues varies with the adequacy of vitamin C nutrition. Vitamin C is necessary for the action of the complements in immunity reactions. Evidence indicates that many humans are in a stage of vitamin C depletion which if present in the guinea pig would be sufficient to render the pig's teeth susceptible to injury by toxins. Doctor King stated that it would be advantageous to administer ascorbic acid in a citric acid solution to decrease destruction in the intestinal tract.

Vitamin D

Edwards A. Park stated that ten forms of vitamin D had been discovered, of which two, irradiated ergosterol and irradiated 7-dehydro cholesterol were of practical importance. Thirty-nine investigations have been reported of the results of administering various forms of vitamin D to infants, all the results not being in agreement. In these clinical observations it is hard to get enough children. Italian infants are susceptible to rickets while Jewish children are nearly immune. As nearly as one can determine, the vitamin D in irradiated ergosterol is as effective an antirachitic for the human as is the D in cod liver oil, rat unit for rat unit. In the human, the maximum incidence of rickets is at 4 months of age, and the administration of vitamin D should be started at 2 weeks of age. In oils, 800 to 1000 international units of vitamin D daily is the lower level of dosage for prevention of rickets. Though any amount of vitamin D which will prevent rickets will eventually cure the condition, we should produce a cure more rapidly. Premature infants may require from 5000 to 10,000 international units of vitamin D to prevent rickets. Vitamin D dispersed in milk is more effectual, unit for unit, than vitamin D in oils. Four hundred international units of vitamin D per quart of milk is better than 135 units. He is thoroughly convinced of the advantages of using milk as a source of vitamin D, provided the milk has been reinforced with a sufficient amount of the vitamin.

The administration of vitamin D should be continued throughout the growth period. It is harmless and certainly improves tooth nutrition. It should be administered to pregnant women, and to individuals who are deprived of being out of doors, such as miners and invalids. There are a number of conditions in the adult where vitamin D administration is indicated. If this does not affect the calcium and phosphorus content of the blood, its use is innocuous. Doctor Park advocated the use of vitamin D substances which also contain vitamin A, since it is just as easy to administer vitamin D plus vitamin A as vitamin D alone. In relation to the cost per unit of vitamin D, cod liver oil is cheapest, percomorph liver oils stand next, viosterol next, and irradiated milks are the most expensive.

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